SOME OBSERVATIONS ON THE HABITS OF PECTEN DISLOCATUS.

B. H. GRAVE.

With the purpose of studying the habits of the scallop, *Pecten dislocatus*, I collected many young specimens ranging from two to ten millimeters in length and placed them in small glass aquaria in the laboratory.[1] They were found in the harbor at Beaufort, N. C., well above the muddy bottom, clinging to eel grass. They were usually attached by several strands of hyaline byssal threads, which were exceedingly strong and elastic.

Although the *Pecten* is so generally known as to make a detailed description of its anatomy superfluous, yet a brief description of certain parts is deemed necessary. For a more detailed study of the anatomy, reference can be made to a paper, by G. A. Drew, on "The Habits and Anatomy of the Giant Scallop."

By reference to Figs. 1 and 2, it may be seen that the shell is rounded and eared. The ears make possible the long, straight hinge line, which extends along their upper borders to their extremities. The right valve is slightly more convex than the left, and near the anterior[2] ear, it has a deep notch. This one feature mars the symmetry of the valves. Between the valves and just beneath the hinge ligament, there is a pad of cartilage-like substance, which is compressed when the valves are closed. It serves to open them quickly when the adductor relaxes (Fig. 3).

The form of the shell and the structure and arrangement of the soft parts within, adapt *Pecten* to the swimming habit. It swims by opening and closing the valves in rapid succession. By varying the position of the mantle so as to control the direction of

[1] Through the courtesy of Hon. Geo. M. Bowers, U. S. Commissioner of Fish and Fisheries, I had the privilege of occupying a table in the Fisheries Laboratory, at Beaufort, N. C., for two months during the summer of 1908. For this privilege and for many kindnesses shown me by the Director, Henry D. Aller, I am glad to express appreciation.

[2] The hinge line is here considered dorsal for convenience in description, although it does not represent the true dorsal of the animal.

the currents of water expelled from the mantle chamber, it is enabled to swim either forwards or backwards, although it usually swims with the opening of the valves directed forwards.

A perfectly symmetrical shell is the form best adapted to swimming, and the presence of any irregularity in it, such as that just mentioned in the pecten, is to be explained either as an adaptation to habits other than swimming, or as a structure, inherited from an ancestral form, and not as yet obliterated through adaptation to the swimming habit.

Although adult specimens were kept in aquaria all summer, no method of locomotion other than smimming was noted, and no clue was gained as to habits which would in any way explain the function of the notch in the right valve. They neither attached themselves by a byssus, nor used the foot for locomotion. Young specimens, however, showed much more activity than the adults, and some observations on their habits are recorded in the following pages.

Concerning the function of the notch in a related species, *Pecten tenuicostatus*, Dr. Drew writes as follows: "I have been unable to satisfy myself as to the function performed by this notch. The sense tentacles on the mantle margin, opposite the notch, are somewhat longer than those adjacent, but I have been unable to determine that they have a special function or that they are especially advantageously placed."[1]

The Sense of Position.

The *Pecten* lies habitually upon the right valve and if placed upon the left, immediately turns over. When lying upon the left valve, it seems to feel the same sort of discomfort which a frog, or other animal with well developed balancing organs, feels when placed upon its back. However, after turning them over repeatedly, they sometimes remained resting on the left valve for several minutes.

The Asymmetry of the Valves.

When dropped through a considerable depth of water, *Pectens* settle about as frequently upon the left valve as upon the right.

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 7.

The slight flatness of the left valve does not serve to make them settle always upon the same side.

The Functions of the Foot.

The foot lies just opposite the notch in the right valve. It appears to be functionless in adult specimens, or rarely used by them, but is made use of to good advantage by the young. Specimens were often seen to extend the foot anteriorly to a remarkable distance, attach it at the tip to the bottom and then, by a powerful contraction, draw the body forwards to the point of attachment. The foot is cylindrical and seems very small to carry such a load; and frequently, after it has been extended and attached, the valves are opened and clapped together, at the same time the foot contracts, the body thus being drawn forward with much less strain upon that organ. This method of locomotion is a combination of swimming and creeping. The force of the current of water expelled from the mantle chamber serves to raise the body and propel it forwards as far as the attached foot will permit. At the same time, the foot contracts and the body lands close to the point of attachment. When this method of locomotion is used, the foot, instead of being extended directly outwards, anteriorly, is usually directed more ventrally, so that the point of attachment is more nearly in line with the force exerted by the swimming movement. Except for the notch in the right valve, this sort of performance would not be possible, because by the closure of the valves, the foot would be crushed.

The foot is, also, frequently used in turning the body over, when placed upon the left valve; it is extended anteriorly from the body and attached; the valves are opened and clapped together vigorously; the body, as a result, is raised and shot forwards, but the weight of the foot and the resisting pull from its attachment cause it to swing over upon the foot as a pivot, the scallop landing upon the other valve, having turned through an arc of 180 degrees.

The above method of turning over, is usually, if not quite universally, used by specimens when placed upon the left side for the first time. After a little handling, however, they become much more irritable, seeming to be excited, and at such times, they

manage to right themselves by one of three methods: Sometimes without extending the foot, they open the valves and clap them together. After one or several trials, the body turns over upon the hinge line as a pivot. The mantle must have played a part in this by expelling currents of water in a direction such as to cause the body to turn over.

At other times, a position on the right valve is gained by one or more short swims, the method being continued until the body comes to rest on the right side. They usually manage to alight upon the right valve after a few trials, and then they become quiet.

The Byssus.

When specimens are allowed to lie undisturbed upon the right valve, they usually become attached by numerous strands of strong byssal threads. A short time only is required for this to take place. They frequently become firmly fixed in from two to five minutes and the threads are sufficiently strong to support a weight several times that of the body of the *Pecten*. The byssal threads pass through the notch in the right valve directly to the support below. They adhere, to some extent, to the shell where they come into contact with it.

So long as specimens are kept lying upon the left valve, they cannot, or do not, attach themselves by the byssus. Since the byssal gland lies at the base of the foot, it is possible that the notch, in the shell opposite it, is a structural adaptation directly correlated with the function of the byssus. At any rate, because the byssal threads extend through the notch, in place of over the edge of the shell, the pull has less tendency to tilt the body than would be the case if no notch were present.

In order for the byssus to become attached to the bottom, it is not necessary for the valves of the shell to be opened, since the attachment of the byssus is frequently accomplished while they are closed. The notch in the shell is sufficiently large to allow the extension of the foot to the support during the process of attaching the byssus. It seems that Dr. Drew has observed this process in individuals of *Pecten irradians*, to quote:

"An individual of *Pecten irradians* placed in a glass dish of sea water will sometimes protrude its foot from the shell, apply it

closely to the bottom of the dish and after a short time, slowly withdraw it, leaving a rather broad band of slightly yellowish material attached to the glass and connected with the foot by the byssal gland. This is not composed of small threads as in the mussels *mytilus* and *modiola*, but it may be sufficiently tough to support the weight of the animal, if, after a few minutes, the dish is carefully turned over."[1]

Summary.

By way of summary, therefore, it might be said concerning the function of the notch that it makes possible a much freer use of the foot and byssal gland, and is in some way connected with the function of these organs. Although many mollusks live in the mud, the fact that young *Pectens* do not is evidence that they do better out of it. The foot and byssus enable them to climb upon supports and maintain their position there. As they approach maturity, they assume more and more the swimming habit and the foot and byssus lose, to some extent or entirely, their functional activity. If these organs are not functional in full-grown *Pectens*, as seem probable, the notch is no longer of any value to them, although it is not obliterated.

The *Pecten* has the sense of position well developed.

Earlham College, Richmond, Indiana,
March 6, 1909.

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 18.

Explanation of Plate I.

Fig. 1. Shows the left valve as it appears from surface view. It is not quite symmetrical.

Fig. 2. Shows the right valve as it appears from surface view. The prominent notch at the base of the anterior ear can be seen.

Fig. 3. Is a view of the inner surface of the right valve. The dotted line shows the position of the cartilage pad which aids in opening the valves.

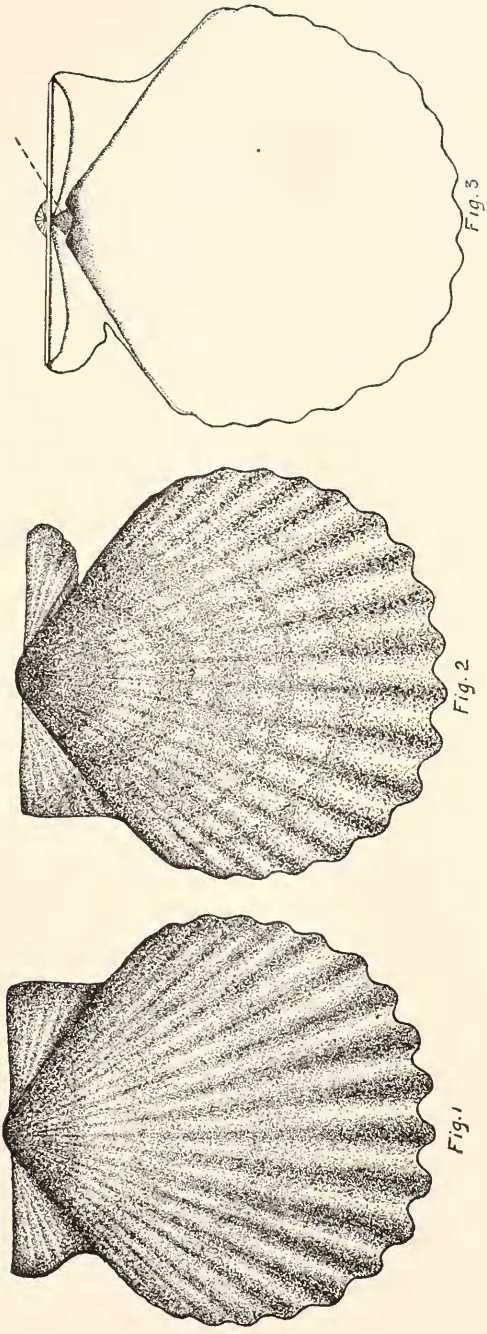
Fig. 3
Fig. 2
Fig. 1

THE DYNAMIC FACTOR IN REGENERATION.

T. H. MORGAN.

With the publication of the data here presented the series of experiments that I have carried out on *Tubularia* for several years may be considered as temporarily brought to a close. I take this opportunity therefore to sum up the evidence bearing on the problem of the formative factors of regeneration, as exhibited by this hydroid. In the course of my experiments tentative hypotheses have been proposed here and there that have at least served to suggest further experiments. The conflicting evidence sometimes inclined me towards one point of view, sometimes towards another; yet, all in all, the same general line of thought, if sometimes vague, can be traced through the attempts to analyze the results. It will be my endeavor here to bring more into the foreground those theoretical deductions that seem to me at present to be best in harmony with the experimental evidence.

That the dynamic factor in regeneration is not primarily the outcome of physiological movements of the animal, or of its parts, is made probable not only by many facts familiar to every student of regeneration, facts that show that the new part often develops under conditions where movement or function in this sense is absent, but also by the important experiments of Zeleny and of Stockard with the jelly-fish *Cassiopc.* They have shown that when one half of the disc is brought to rest by removing the sense organs (and scratching a barrier zone across the connecting ectoderm) the quiescent half regenerates as well and as rapidly as the half left pulsating during the period of regeneration.

Since every part of the stem of *Tubularia* is capable of producing a hydranth the inhibition of basal development is obviously due to the presence of a hydranth or of a developing hydranth at the oral end. Two alternative chemico-materialistic explanations have been suggested for cases like this. (*A*) The oral hydranth may use up some materials necessary for the formation

of a basal hydranth. (*B*) The hydranth may produce some materials that inhibit the development of other hydranths from the remainder of the piece; hence the inhibition, as long as a hydranth is present or developing. On first thought these alternatives would seem to cover the only possible ways in which the problem of regeneration may be presented — at least as long as the problem is confined to purely physiological actions of a chemical order. There are, however, not a few considerations indicating that the fundamental interpretation may lie in a different conception of the problem. I shall try here to emphasize this other point of view without attempting to develop it into a theory of regeneration. At most we may hope at the present time to find in the facts some indication of the nature of the problem if not its entire elucidation.

A number of experiments have been made that seem to indicate that the temporary inhibition of the development of the basal hydranth in *Tubularia* is not the result either of the using up of materials by the oral hydranth, or of the setting free of inhibitory stuff. The simultaneous development of hydranths at both ends of a piece, which frequently occurs in short pieces, is a case in point. Both ends develop at the same rate as when a single hydranth develops, and not half as fast as the hypothesis demands. Again the development of a basal hydranth does not appear to inhibit the oral development as we should expect if the result were dependent simply on the presence of materials in the stem. Some experiments of MacCallum's with plants have an important bearing on this point. If the terminal bud of the bean is removed, the buds in the axils of the cotyledons develop. But if the activity of the terminal bud is simply lessened by inclosing that part in an atmosphere of hydrogen, the basal buds do not develop. Hence the result is due not to activity of the terminal end, but to its presence or absence. In a different way the same fact is brought out. A piece of willow stem is cut off, its middle third is inclosed in a tube filled with moist air, so that the buds in this part are encouraged to begin their development; the dry air retarding the development of those outside. After the middle buds have unfolded, the entire piece is inclosed in a moist chamber, when the more apical buds sprout forth, while none

of the buds basal to the middle region develop. The presence of growing shoots in the middle of the piece does not inhibit the apical buds from developing, if external conditions are supplied favorable to their growth, but the basal buds are inhibited by the presence of shoots on the more distal parts. These facts are incompatible with the assumption that the results are due to the presence of materials used up by those parts that develop first to the exclusion of other parts. They also show that the alternative view is untenable, for, the presence of growing shoots in the middle of the piece is not antagonistic to the development of shoots in other regions provided those regions are situated more distally.

In the case of *Tubularia*, it is more difficult to present convincing evidence that distal hydranths do not produce materials inhibiting the development of basal hydranths, improbable as such an interpretation may now seem. But the fact that basal hydranths do develop after the oral hydranths have formed may seem to discredit this view. Here, however, an apparent paradox is found. The experiments seem to show that when the oral hydranths develop, the basal hydranths are retarded in development, but they do develop later, and the results also show that if both start simultaneously both develop at the normal rate. The paradox is due, I think, to two antagonistic factors at work at the same time. Admitting that the oral development tends to inhibit the beginning of basal development, we also find that if other influences suffice to start both simultaneously, the on-rush, so to speak, of the process once begun changes the conditions that tended to prevent the starting. Strange as this seems it is little more than a statement of the facts. The same results may be put in a somewhat different way. A cut end being present, whether oral or basal — the conditions that call forth hydranth formation are given. Experiments show that the oral end tends to develop first, its development acts as a partial inhibition of the basal hydranth-formation. If this influence is strong enough the basal development is temporarily held in abeyance, but if not the inhibition is overcome. Once overcome, the formative influences do not check the further action of the basal end. In this connection it is curious to note that small oral pieces produce simultaneous hydranths more often than larger

pieces. The interpretation of this seems to be that the tendency to produce hydranths, both oral and basal, is stronger near the distal end and decreases basally. In short pieces the sensitiveness of the two ends to those influences that call forth the hydranth is so great that both ends develop simultaneously or nearly so, hence the oral end has not time to get a sufficient start over the basal to stop its development. It should be noted in passing that it is probable that the influence preventing basal development is not only the oral development, but a direction-factor present in the stem at all times. This factor we call polarity. The interesting point is that this factor seems to be more capable of inhibiting basal hydranth formation when an oral end is developing than when such development has not yet begun. The basal development, however, does not appear to delay the oral process. It is acting against the polarization and its influence is less felt throughout the stem, as experiments by Stevens and myself have shown. These considerations lead, I think, to the view that the essence of our problem lies in that peculiarity of the piece that we designate its polarity, and not in the absence or presence of formative substances in Sach's sense.

If our analysis is correct, we are led to look upon living material as possessed of a certain formative principle that has so to speak a "sense of direction." The next step will be to study the nature of this principle and see what properties we are justified in ascribing to it; for while it may be beyond our powers at present to state precisely the nature of the directive principle, we may at least be enabled to work out its manifestations. Some of these manifestations become apparent in the study of the regeneration of *Tubularia*. One of its most striking modes of action is seen in the inhibition of basal-hydranth formation. Most interesting is the result that its action becomes intensified by developmental processes going on at the oral end, as shown by the fact that if the oral development is suppressed by tying that end, the basal development is much accelerated. It is accelerated in the sense that basal hydranths more often develop at once than when both ends are open, but not in the sense that the basal development is faster than when this end also gets as early a start as the oral end. In other words, there is no speed-

ing up of hydranth formation as such, but the initial inhibition is overcome.

The special problem with which this paper deals is the nature of what takes place at the basal ends when the oral end is kept open and when it is tied. Is the retardation of such a kind that a slower process of development is going on at the basal end while the oral end is developing, or does the basal end not really begin to develop until the oral end has formed its polyps. If so, what gives it its start later? The following experiments were devised to study these questions.

Experiment I. — The purpose of the experiment was to determine whether when both oral and basal ends of a piece are left open constructive changes are slowly going on at the basal end. Some pieces were cut off and left open (*A*); later other pieces were cut off and the oral ends tied (*B*) and at the same time the oral ends of (*A*) were tied. It was found that the basal ends of the (*A*) pieces did not develop faster than those of the (*B*) pieces, showing that the changes at the basal end of (*A*) are not progressing, but are held in check by the developing oral hydranth.

Control I. — In some pieces the old hydranths were left intact and the pieces cut off. No basal hydranths began to develop until the old heads began to be absorbed. The presence of the old heads inhibited the development of the basal hydranths until the heads had degenerated when the latter appeared.

Experiment II. — In order to find out whether, when the oral end is tied, changes take place throughout the piece that tend to make more rapid the development of basal hydranths, or whether these changes are localized at the basal end where the new hydranth develops, the following experiment was tried. The oral ends of many pieces of the same length were tied. Then after several hours' interval differing in several experiments, the basal end was cut off, (*a*) just inside of the area that would form the basal hydranth, (*b*) in the middle of the piece, (*c*) just below the ligature. In general the development of the basal hydranth was delayed as compared with control pieces tied but not cut off at the basal end; the delay was the greater the further removed the cut from the basal end, despite the fact that oral levels tend to regenerate faster than more basal levels. The differences

are more apparent the longer the time that elapses before the basal pieces are removed. The differences are not very marked at the different levels indicating perhaps that changes take place throughout the piece and not only at the basal end although more pronounced in the latter. The different levels of the cuts make it difficult to ascribe the results solely to the general changes in the piece, for the more orally situated cut ends have an advantage in level as other experiments have shown.

Experiment III. — Pieces were cut off at the same oral levels. After 23 hours the hydranth region at the oral end was cut off of some pieces (*A*), others were cut in two in the middle of the piece (*B*), and for a control some pieces were left as before (*C*). A slight retardation occurred after another 12 hours in (*A*), less in (*B*) as compared with (*C*). Removal of the hydranth forming region after 23 hours causes delay but the delay is not so much as though a new hydranth had developed at the new cut, showing that changes directed towards hydranth formation are going on not only in the region where the hydranth will develop but at more basal levels as well.

Experiment IV. — This experiment was like the last, except that the basal ends of all the pieces were tied, thus preventing the basal end from exerting any influence on the result. Other experiments had shown, however, that the basal development, even if it occurs, has apparently no retarding influence on the oral development. The results, as was to be expected, were the same as in the last experiment. It is interesting to note that in both the influence of the cutting causes a greater delay in the first appearance of the primordia of the hydranth than on their later development; for later the differences seem to be less than at first. This may be due to an acceleration extending throughout the whole time that is more effective after a beginning has been made than before the start.

Experiment V. — Some previous experiments had left undecided the question whether, when the oral end is left open for several hours and is then tied off, the basal development is more rapid than when the oral end is tied at once. If such an acceleration really occurs it might seem to indicate that changes take place in the oral end that produce accelerating materials even for the

basal ends. I was particularly anxious to settle this point definitely, for obviously, if such acceleration could be proved, it would furnish evidence in favor of a chemical process, especially since other experiments had seemed to show that the basal end does not begin its development when both ends are left open. I have carried out rather an extensive series of experiments that give, I think, a definite answer to the question.

When pieces are left open at both ends from four to nine hours, and are then tied at the oral end, the basal development is slightly retarded as compared with its development in pieces tied at once. There is little evidence in favor of the view that the later tied pieces can make good the loss of four to nine hours, and of course they can not catch up if a longer time elapses. Whether they may do so in later stages is more difficult to decide, but this does not concern the main point here raised.

Individual differences in rate, differences in stems, and uncontrollable differences in level tend to obscure results that depend on only four, six, and nine hours differences in start. The above statement holds, therefore, only for average results. There was found no evidence in favor of actual acceleration, whether there is some relative acceleration is difficult to decide. If the hydranths do not develop promptly *i. e.*, if a long time elapses between the tying and the appearance of hydranths, the initial differences of a few hours may be lost.

Experiment VI. — Another attempt was made to see whether changes take place in the piece as a whole, after it is cut off, that make more rapid the development of an *oral* hydranth when a new cut is made.

Pieces were removed and after four hours somewhat more than the oral hydranth region was cut off. In some cases the newly cut ends developed as fast as did the hydranth in the small pieces cut off, but the latter may have been retarded by the operation or by the smallness of the pieces ; yet in some cases the development of the newly cut ends was as rapid as in control uncut pieces. This result indicates that changes take place in the pieces behind the actual region of hydranth formation that lead toward the development of a hydranth.

Experiment VII. — In this case pieces first cut off were after

ten hours cut in two in the middle. Comparing the rates of development of the oral ends of the oral halves with that of the oral ends of the basal halves it appears that the latter are slower, but there is evidence that the retardation may be somewhat less than the ten hours difference in initial start. The basal halves in this experiment are also somewhat behind the basal halves in the last experiment, which seems to show that the general changes in the excised pieces that go towards oral development decrease from the cut ends inwards.

It has not seemed necessary to give the details on which these general conclusions are based. The nature of the case makes it difficult to obtain results as definite as one might wish, despite the precautions that were undertaken to make the conditions as uniform as possible. The general conclusion that changes take place in the piece as a whole, after its removal, that are in the direction of hydranth formation, seems fairly certain. Less certain perhaps is the evidence to show that when the oral end is tied similar changes take place in the pieces that accelerate basal development in regions beyond the hydranth forming region, but this conclusion too is, I think, quite probable. The nature of these changes is not revealed.

The Dynamic Factor in Egg-Development.

Students of the processes of regeneration have without exception made use of the term polarity to express a directive factor observable in their results, and to this factor is sometimes ascribed an active rôle as a controlling influence, at other times the term is used descriptively merely as a statement that the new structures are directed in the same way as the part removed. In both respects the word has been useful, however vague our conception of what polarity may be. Our analysis of the process has now gone sufficiently far, I think, to justify us in an attempt to come to closer quarters with the term. Without reviewing the opinions that have been expressed as to the nature of polarity, I shall try to contrast two views of its nature that seem to me to represent the two main lines that speculative thought has followed. It should be noted that the term is used equally by students of embryology and by students of regeneration. The former

finds axial relations in the egg — polarity, bilaterality, radial symmetry, etc.; the latter finds the new organs regenerated in definite relations to the old. To some observers the distribution or the stratification of the materials of the egg, has seemed a sufficient basis for the results referred to under the term polarity; to others it has seemed more probable that there exists in the egg an arrangement or structure that has axial relations from which result not only the depositions of the formed materials but also the nature of the action of the parts. Polarity is from this latter point of view not simply a passive structure, but a relation of the parts that directs the shifting series of changes that we call development. At one time one of these views has seemed more probable; at other times the other. The history of modern experimental embryology and regeneration shows the influence that these views have had on those who have followed the new work. In a general way the two views may be classed as the materialistic or chemical and the dynamic or physical conceptions of the developmental process. At present it seems to the writer that the evidence has been steadily pointing to the second of these contrasting views as the more probable. As far as the egg is concerned, the recent experimental work goes to show that the visible inclusions of the protoplasm (yolk, oil and other granules perhaps) are not the fundamental causes of the formative processes, although they may be needed in certain regions to carry out the future development of the structures that there appear. In regard to regeneration it has been evident for some time, that the specification or the differentiation (with its concomitant products), cannot be unreservedly utilized as a basis for an explanation of formative processes that take place. For example, if the gross materials or the differentiations of the head end of a planarian are the causes of that region being a head, it is inexplicable that when the head is removed it could regenerate a tail. There must be something else behind what we see that is responsible for the change that takes place. These and other considerations lead to the view that there exists a fundamental property of living matter that is the formative principle of development. On two former occasions, when attempting to analyze the results of regeneration in *Tubularia*, the author tried to account for the re-

sults of polarity on the basis of a stratification of the materials. Influenced at the time by recent results in experimental embryology that seemed to show that visible substances of different kinds in the egg are really responsible for the development of its parts, the same idea was applied to the problem of regeneration, despite the fact that I had on more than one occasion rejected the hypothesis of formative stuffs, in Sach's sense, as sufficient to account for the facts of regeneration. Yet a careful reading of the papers here referred to will show that I still held, though perhaps not always consistently, to the conception that back of these differentiated materials lay the real differentiating factors.[1] It now seems to me that the evidence, which at that time seemed so strongly to favor the idea of the importance of the grosser materials of the egg, is insufficient to establish its case, and that the important factors of development are dynamic properties of the bioplasm, rather than the formed products of the egg, or of the differentiated products of the adult animal. This statement does not mean that the visible products in the egg play no rôle in development. The evidence still shows that they may do so, but their rôle seems to be secondary, not primary.

The interrelation of the parts seems to be one of the most evident expressions of the fundamental formative influences. Several years ago a consideration of a number of results in regeneration led me to state that this relation might be expressed as a sort of tension. This view has been objected to on the ground that it does not appear to explain the matter any better than before. In a moment of doubt and in order to give the

[1] One further word of explanation. The rate of hydranth formation varies with the distance of the cut end from the original hydranth. I have spoken of this difference in rate as explicable on the assumption of the hydranth-forming materials decreasing toward the base, *i. e.*, away from the hydranth. It was unfortunate to have used the term hydranth materials, although I made sufficiently clear in the text that I did not mean to invoke the stuff-hypothesis in this connection. It is not entirely clear on what the difference in rate depends; most probably on the stem being less specialized as a store-house of food substance nearer the hydranth; probably also on some difference connected with the thickness of the walls with which the specialization may also be connected; possibly neither of these but some more fundamental characteristic is responsible for differences in rate. In any case it is not obvious that there is any connection between this difference in rate and the polarity of the piece. The latter is the same for all levels — the time it takes the piece to be remodelled seems to be referable to something else.

statement a meaning for those who believe no suggestion to be of value unless it refer the problem to ordinary properties of inorganic bodies, I suggested that osmotic pressure might be the cause of the tension differences in the parts. This was an unnecessary concession. The behavior of fluid crystals (according to Lehmann) shows that the formative changes can be accounted for on the basis of a tension exhibited by the molecules of the substance of the crystal. While the organism may not be put down as a fluid crystal, still we see that physical properties other than osmotic pressure and surface tension may play an all-important rôle in form-changes. It may be that a similar property is the cause of the formative changes in the organism. In any case, the facts that I had in mind suggested that tension of some sort is an important dynamic factor in development, perhaps *the* important factor. The facts still seem to me to indicate some such relation between the parts, and no one regrets more than I that we cannot "explain" the results even if my suggestion prove to be in the right direction.

Still later a consideration of certain facts of development led to the suggestion that two known properties of the organism — contractility and irritability — also play a very important rôle in embryonic and regenerative development. I shall not attempt to review here the argument which led to this point of view. How far and in what sense contractility and irritability are better expressions of the tension hypothesis, it is not easy to state. So far as contractility is concerned Lehmann's recent important paper on "Scheinbarlebende Kristalle"[1] shows the possibility at least of referring this property also to a condition of molecular tension. We are still too ignorant of the physical basis of irritability to make speculations in this direction profitable, but it may be well not to lose sight of this property of living matter in our attempts to analyze further the problem of development.

Stereometry of the Bioplasm.

Polarity implies difference in one direction. Every student of regeneration knows that in all three dimensions of space the same factor is present. Polarity is therefore only a part of the problem,

[1] *Biol. Centralb.*, XXVIII., 1908.

and so far as it draws attention away from the whole problem it seems best to substitute the term stereometry.

Sufficient evidence has accumulated, I think, to show that stereometry has a dynamic side — in so far as it is a result of the molecular factors that determine the relations of the parts to each other. A question of fundamental importance here presents itself. If the formed substances at each level are the products of the bioplasm, must not the bioplasm itself be stratified in nearly the same sense? It was this idea that I had in mind when I wrote in 1906: "If we imagine a stereometric network as a part of the specialized structure, we must be prepared to admit that it changes at each level as the structure changes. Therefore it seems to me simpler to base our hypothesis of polarity on the difference in differentiation itself, and not on an imaginary polarized system associated with the living materials." But the point I overlooked was that there is no need to suppose that a heterogeneous network of bioplasm exists because the visible structure formed by it is different. The relation of the polarized material to the ends of the material (indeed to all its directions) suffices to account for the difference of level. In fact if the stereometry rests on a dynamic and not a statical relation of the parts this is the logical standpoint.

It has been suggested that irritability may be related to the dynamic factor of development.

The effects of irritability at any level may be realized through the chemical changes inaugurated. These chemical changes once started may, if enzymatic, thenceforward continue (unless checked by other chemical processes), independently of the factor that set them going.

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THE REGULATORY CHANGE OF SHAPE IN PLANARIA DOROTOCEPHALA.

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The recent discovery of *Planaria dorotocephala* Woodworth (Woodworth, '97) in very large numbers, near Chicago, has made it possible for me to use this form for extensive series of experiments. The species is very similar to *P. maculata* in structure, behavior and regulation, but possesses some advantages over that species for experimental work. It attains a larger size, is more active, and can be obtained in unlimited numbers and all ages in this locality, while *P. maculata* is much less abundant. I found the same species in California some years ago (Child, '06), but was unaware at that time that it had been described.

In the present paper only certain experiments concerning the effect of anæsthetics on form regulation will be considered. It is possible by the use of dilute solutions of anæsthetics to control, modify and inhibit various regulatory processes almost at pleasure. For example, head-formation can be made a process of redifferentiation instead of regeneration in almost any desired degree (Figs. 14 and 16) or can be completely inhibited, according to the conditions of the experiments, and the same is true concerning the formation of a new posterior end and a new pharynx, and the regulatory changes in the intestinal branches. Moreover, the use of anæsthetics permits, in greater or less degree, an analysis of some of the various factors concerned, and finally, it is possible by this means to produce individuals capable of continued existence if returned to water which possess characteristics, or perhaps more properly, combinations of characteristics which do not occur in nature.

The anæsthetics chiefly employed in my experiments thus far are

alcohol, ether and acetone-chloroform, commercially known as chloretone. The effects of all are essentially similar in kind, but of course differ quantitatively according to the substance and the concentration. Ether, for example, in a concentration of 0.4–0.5 per cent. produces about the same effect as alcohol in a concentration of 1.5–1.6 per cent. In solutions of these concentrations, individuals and pieces have been kept alive as long as three months, though the resistance differs greatly according to the condition of the animals and various other factors, most of which can be controlled experimentally either directly or indirectly.

In order to avoid as far as possible decrease in concentration of the solution by evaporation the following method has been used. The animals or pieces are placed in Stender dishes of several hundred c.c. capacity with ground edges and a cover with ground groove exactly fitting the edge. The groove is filled with solution of the same concentration as that in the dish so that the dish is sealed so long as the fluid does not evaporate from the groove. After the dishes are thus closed they are placed in larger jars containing a liter or more of the same solution and these are sealed with vaseline and the covers weighted so that no escape of vapor or entrance of air is possible. And finally, all solutions are renewed every forty-eight hours and are made up immediately before using. In this way it is possible to compare the effect of the anæsthetic upon pieces of different size and from different regions of the body and also upon worms in different physiological condition.

This method makes possible the control and modification of form regulation in this species to a greater extent than any other which has been described. At present, however, only certain points will be considered, a full account of the experiments being postponed to a future time.

In several of my "Studies on Regulation" I discussed the changes in shape and proportion which occur in isolated pieces of various species of turbellaria and which, under the usual conditions, constitute an approach to the shape and proportions of the whole animal. These experiments with anæsthetics furnish new data which confirm my earlier conclusions, and it is some of these data which are to be presented here.

I. Experimental Data.

When whole individuals or pieces are placed in 1.5 per cent. alcohol or in 0.4–0.5 per cent. ether they lose the power of coördinated movement almost entirely for a time. After four to five days, however, they become in some degree acclimated to the new conditions and begin to move about very slowly, but with increasing vigor as time goes on, though they never attain the normal motor activity. Pieces including the old head begin to move about earlier than pieces without a head, for the latter must form a new head before they can regain the usual degree of motor activity, and regulation is greatly delayed in the solution. The important point for the present purpose is that for some days movement, and particularly coördinated locomotion, is almost completely eliminated. It is of interest to determine to what extent regulation occurs under these conditions.

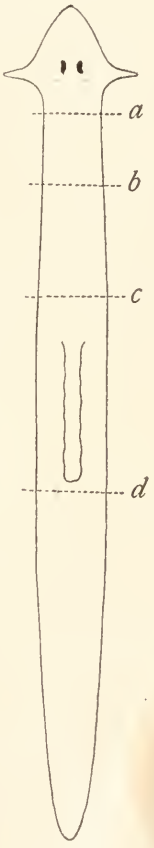
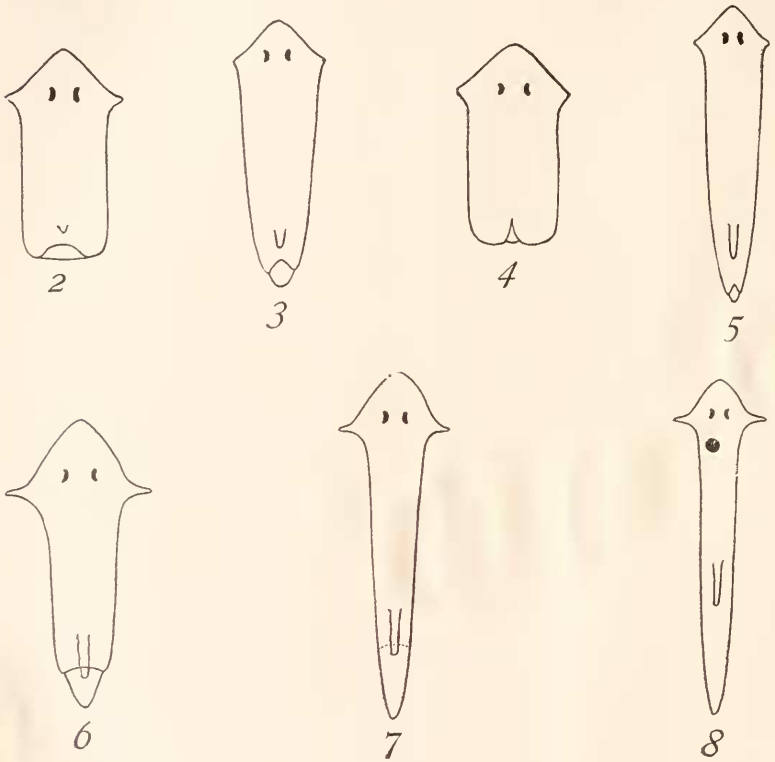


Fig. 1.

The first experiment to be described concerns pieces including that part of the body anterior to the line *b* in Fig. 1, *i. e.*, short pieces with the old heads. In Fig. 2 a piece of this kind after ten days in 1.5 per cent. alcohol is shown: during this time the piece has moved about but little and that chiefly during the last few days. Fig. 6 represents a similar piece after five days in water, Fig. 7 the same piece after ten days. Comparison of Figs. 2 and 7 shows that regulation in the alcohol is greatly delayed: a little new tissue has been formed at the posterior end, but, as a microscopic examination under pressure shows, it is still a mass of cells without any marked visible differentiation, and it can readily be seen that it is not used as a tail and is not attached to the substratum as the animal creeps slowly about; a small group of cells is present in the pharyngeal region, but these likewise show no marked differentiation. In water, on the other hand, a new tail has been formed which functions in the normal manner, contracting, extending and attaching to the substratum as the animal

creeps; the pharynx is well-developed and sections show that it possesses essentially the same structure as the pharynx in uninjured animals.

But the difference between the two pieces is most marked as regards change of shape. The piece in alcohol has not elongated at all, in fact it has decreased in length and it may be noted incidentally that the "auricles" on the sides of the head are greatly reduced. The piece in water (Fig. 7) has in the same



FIGS. 2–8.

length of time elongated to nearly twice its original length, has become much more slender and tapers posteriorly. This piece has moved about during regulation to an even greater extent than the uninjured animal, for short pieces with the old heads are usually more active than whole individuals.

After ten days the piece in alcohol gradually becomes more

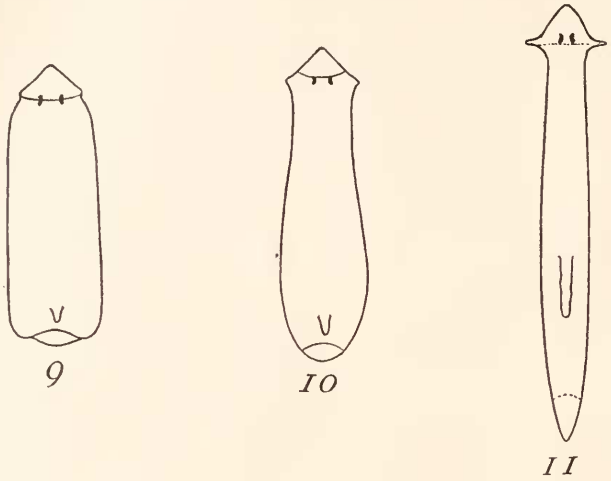
active, though it never attains anything like normal activity. At the end of twenty or twenty-five days it has acquired a shape like Fig. 3. The posterior end now functions as a tail to some extent and attaches itself to the substratum as the animal advances but the amount of new tissue has not increased. The piece may live for six weeks or more in alcohol but it never undergoes any appreciable further change in shape. The newly formed parts undergo some degree of differentiation but never attain the characteristic adult structure. Apparently the piece has attained approximately a condition of equilibrium for the conditions under which it is living.

If the concentration of the solution is gradually increased after the first three or four days it is possible to inhibit practically all regulation beyond the closure of the wound: the pharynx does not appear, no further growth of new tissue at the posterior end occurs, and the piece undergoes no elongation (Fig. 4). Under these conditions the piece does not acquire the ability to move about.

If now these pieces which have attained equilibrium in alcohol be returned to water they gradually resume the process of regulation, but with certain differences from pieces which have not been in alcohol. The chief difference for present purposes is that the outgrowth of new tissue at the posterior end does not proceed until it reaches the usual amount. The tail is formed almost entirely by a redifferentiation of the old tissue (Fig. 5). The pieces may undergo change of shape after their return to water until they attain practically the same shape as pieces which have not been in alcohol. Fig. 5 shows a later stage of Fig. 4 after its return to water and Fig. 8 will serve as regards shape for the late stages of either water or alcohol-water pieces.

These results, which are merely illustrations of what I have observed in several hundred pieces, permit certain conclusions of interest. In the first place it is possible to inhibit entirely the change in shape without inhibiting entirely the processes of redifferentiation and regeneration, and the change in shape can be stopped at any point without stopping entirely other regulatory processes (Fig. 3). On the other hand, if the growth of new tissue from the cut surface is inhibited in earlier stages (Figs. 2

and 4) the change in shape may occur in later stages (especially after return to water) without the formation of more new tissue (Figs. 3 and 5). It follows that the factors determining the change in shape must be in greater or less degree different from those determining the localization and growth of new parts. Moreover, the change in shape occurs only when the piece is capable of locomotion and it is in general proportional to the locomotor ability of the piece.

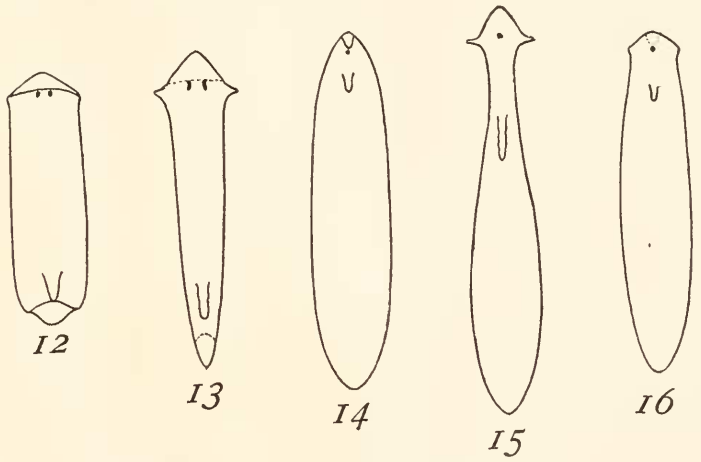


FIGS. 9–11.

But pieces which do not possess the old head afford even more positive evidence for these conclusions. Fig. 9 represents a piece corresponding to the region between the lines *a* and *c* in Fig. 1 after fifteen days in 1.5 per cent. alcohol. A new head has appeared, a small new pharynx is present as a mass of undifferentiated cells, and some new tissue has formed at the posterior end, but the piece as a whole shows no approach to the normal shape : it has undergone no marked changes in proportion. Incidentally it may be noted that the pharynx in such pieces appears much further posteriorly than in similar pieces in water. After seven days more the piece has the shape shown in Fig. 10. It moves about slowly, but its movements are different in character from those of pieces possessing the old head : here the posterior half of the body is very evidently not under complete control,

i. e., is not fully coördinated with the anterior region, and when the animal advances it is simply dragged along as a mass of inert material, its posterior end being only very rarely attached to the substratum. The shape of the piece suggests that the anterior part is being stretched by the strain upon it of the posterior portion, and I believe that is exactly what is occurring.

Similar pieces in water attain in the same length of time the shape and structure shown in Fig. 11. Pieces in alcohol of 1.5 per cent. do not change in shape much beyond the condition shown in Fig. 10, but if they are returned to water they regain their normal locomotor activity and may finally reach a shape like that of Fig. 11.



FIGS. 12–16.

In these cases a new head, a small new pharynx and some new tissue at the posterior end have appeared without any marked change of shape in the piece as a whole (Fig. 9). Evidently the change of shape and the localized formation of new tissue are not necessarily correlated.

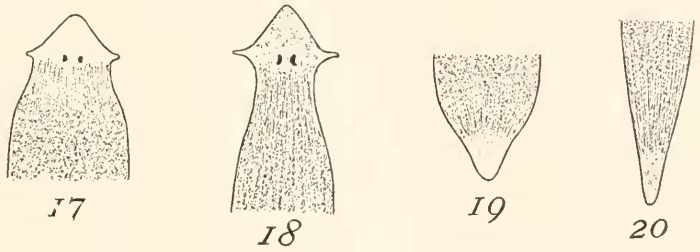
The same thing appears in Fig. 12, which shows a piece from the same region (*a* to *c*, Fig. 1) after fifteen days in 0.5 per cent. ether. Head, pharynx and posterior end have formed but no change in shape has occurred. This piece was returned to water at this stage and after seven days more had attained the shape shown in Fig. 13.

In another series pieces comprising the whole post-pharyngeal region were used (posterior to *d*, Fig. 1). These pieces, after eighteen days in 0.5 per cent. ether, had attained the condition shown in Fig. 14. A small head is forming, almost entirely by redifferentiation, at the anterior end, with one median eye, and a small pharynx is present. At this time half of the pieces were returned to water and half remained in ether. After nine days more the pieces in water had acquired the condition shown in Fig. 15 while those in ether were like Fig. 16. In the pieces returned to water the anterior half is greatly elongated but the posterior half remains much as before. In these pieces, as in the one described above, the posterior part was dragged about by the more active anterior portion. Gradually complete coördination returned and the posterior end began to attach itself to the substratum as the animal advanced and after this the shape gradually approached that of the normal animal.

Further data along this line could be presented but these cases are sufficient to show that it is possible to delay or inhibit the change in shape to any desired degree, and to induce its occurrence at any time. Moreover, the manner in which it occurs can be controlled and altered indirectly by using pieces of different sizes and from different regions of the body.

To my mind the evidence indicates very clearly that the change in shape is primarily a mechanical deformation of the body in consequence of the altered direction of the strains to which it is subjected as the animal advances. To control the change experimentally we have only to control the locomotor activity. In several of my earlier papers (Child, '02, '03, '04*a*) I have described the method of locomotion in certain species of turbellaria: in *Planaria* longitudinal strain arises in essentially the same manner as in the other forms discussed, the use of the posterior region as an organ of attachment being one of the chief factors. Moreover, there is considerable direct evidence that the tissues of a region undergoing change of shape are being stretched. In regions where the decrease in width and the elongation of the old parts begin, the chromatophores are always greatly elongated, and their elongation is greatest where the change in shape is most rapid. As the change goes on they become drawn out into long

lines. Figs. 17 and 18 indicate this change in shape of the chromatophores in the region posterior to a new head, and Figs. 19 and 20 for a region anterior to a new tail. In the pieces in alcohol and ether this change in shape of the chromatophores appears only when the change in shape occurs, not when the new tissue is formed. In cases where the change of shape is inhibited in the anæsthetic (Figs. 2, 4, 9, 12) it does not appear at all, but if such pieces are returned to water, and the change of shape occurs, the stretching of the chromatophores also appears. In Fig. 14, for example, it did not appear so long as the piece was kept in the ether, but after several days in water it was most conspicuous in the slender anterior region (Fig. 15), this region appearing almost



FIGS. 17–20.

as if finely striped in the longitudinal direction. This change in shape of the chromatophores is actually a stretching, not a migration, for it is possible to select some particular spot which happens to be conspicuous for some reason and to observe its change of shape from day to day: in such cases it can be seen clearly that merely elongation not migration occurs.

A similar elongation is visible in the parenchyme cells in section. Stevens ('07) has recently described this elongation or orientation of the parenchyme cells and regards it as indicating migration, but Steinmann ('08) does not agree with her. As a matter of fact the specimens in which the change of shape is inhibited by anæsthetics show nothing of the sort even in regions adjoining those where new tissue is being formed, but if such pieces be returned to water the cells of the parenchyme become very distinctly elongated or oriented in the direction in which elongation of the body is occurring, even though no new tissue is being formed at the time. In short the change in shape or

arrangement of the parenchyme cells probably has nothing to do with active migration, but merely indicates the direction of the strain which produces deformation.

These histological features then, support and confirm the other observations, and all appear to show that the change in shape is primarily a deformation in consequence of strain rather than a complex physiological process.

There can be no doubt, however, as I have repeatedly pointed out that reactions of various kinds result from the strain and deformation: muscles and other tissues undoubtedly "adapt" themselves to the new relations of parts. In fact there is no apparent reason why the change of shape should not continue indefinitely, or at least until the elasticity of the tissues became involved, if nothing but the mechanical deformation took place. Undoubtedly "functional adaptation" to the altered strains occurs and this determines how far the change shall proceed. Sooner or later the tissues adjust themselves fully to the new conditions, *i. e.*, a condition of equilibrium is attained and change in shape ceases. Under the usual conditions this gives what we commonly call the "normal" shape, but I have shown above that under other conditions the shape may be different. No one would deny, I suppose, that the shape of cells is determined in many cases (*e. g.*, the polyhedral shape of blastomeres in many eggs, the polygonal outline of many epithelial cells, etc.) to a greater or less extent by purely mechanical factors. If this is the case it seems scarcely possible to deny that purely mechanical factors may be concerned in determining the shape and arrangement of parts in animals without hard structures and with tissues of a high degree of physical plasticity. That they are the only factors in such cases, or that because they are factors in some cases, they must be in others, I certainly do not and never have maintained.

In *Planaria* the width of the head is apparently an important factor in determining the width of the body behind it. The head itself is not involved to any appreciable extent in the change of shape. It furnishes, so to speak, a fixed point, or rather region at one end, and between this and the posterior end the change of shape occurs. Consequently, in short pieces, where the new head is small, the change of shape is very much greater than in

long pieces, where it is much broader. In short pieces possessing the old head and consequently capable of rapid and frequent locomotion, the change occurs much more rapidly than in other pieces and the body often becomes much more slender proportionally than that of the "normal" animal and tapers more toward the posterior end. In pieces from the posterior region of the animal the chief region of attachment forms the largest part of the piece. Since attachment occurs by the lateral margins as well as by the tip of this region, the change of shape occurs most rapidly in the regions just behind the new head, for these are the regions where the greatest change in the direction of the strains occurs.

Summing up the data presented, the first point of importance is that no necessary connection exists between the change of shape and the redifferentiation or regeneration of parts: the two processes can be separated from each other almost completely in time, and are often separated in space. Secondly, it is possible to control the change of shape, to inhibit it, to stop it at any point, or to allow it to proceed, by controlling the locomotor activity. And finally, the tissues in regions undergoing change of shape show very distinct indications of physical deformation in the direction of the strains. The conclusion which seems to me to accord most closely with the facts as they now stand is that the change of shape is primarily a mechanical deformation resulting, at least in large part, from the strains which arise in locomotion, these strains being altered in direction and amount in pieces as compared with the whole animal. The final shape, *i. e.*, the cessation of change is probably determined by the physiological reaction of the differentiating or redifferentiating tissues to the altered conditions, and the consequent establishment of an equilibrium.

II. Function, Form and Regulation.

Since the appearance of my earlier papers on regulation certain reviews and criticisms of the work have appeared. From some of these, particularly from Driesch's review (Driesch, '05) I can only conclude either that I have failed to state my position clearly, or that the reviewers have not become sufficiently acquainted with my work to understand what that position is. Driesch, for example,

has imputed to me certain views which I have not only never held, but which I have expressly repudiated more than once. And recently, in a brief reference to my work, Morgan ('07, pp. 373–4) has cited certain conclusions as mine, which are very different from those which I have reached. I have considered Driesch's criticisms elsewhere (Child, '07) and shall refer to these and other criticisms only incidentally. But a brief statement of my position with regard to certain features of the problems of form and regulation and with especial reference to mechanical factors, movement and use of parts and function in general seems desirable in connection with the new facts presented above.

1. *The Relation between Function and Form.*[1]

In a fully developed organ certain processes occur which are concerned with the maintenance of the organism as a whole. These processes may affect either the relations of parts to each other or the relations of the organism as a whole to the external world, or both. They are commonly designated as functions, *i. e.*, the adult organism may be regarded as a complex machine, which works or functions in a characteristic manner.

But the functions characteristic of the adult organs do not appear in development until a certain stage is reached and the organ possesses a certain structure. Apparently then, development up to the stage where function in the above sense begins is a process of machine-building. Roux's distinction between a formative and a functional stage in development expresses this idea.

But how is the machine constructed and what is the agency which constructs it? The material of which the machine is to be made must be acted upon, arranged, transformed, localized, differentiated, etc. Evidently this point of view necessitates the assumption of a special formative agent or agents of some kind. How shall we conceive this formative agent? Pangenes, determinants, formative substances, entelechies are some of the answers which have been given to this question. All these answers are much alike in that they regard the construction of the organism as a process analogous in some sense to the construction of a

[1] Cf. Child, '06*a*, p. 402, '07.

machine by human agency. When the machine is constructed it begins to function.

This point of view seems to me to be essentially naive and anthropomorphic : moreover it is responsible for certain "Scheinprobleme" which have arisen from time to time, and for certain barren lines of research, particularly in zoölogy, which has been dominated by hypotheses of this general character to a much greater extent than botany.

To my mind life is inconceivable without some sort of function in some sort of structure ; a living structure functions as long as it is alive ; its function is its life. If this view is correct, the organism is functioning in some manner at all stages of its existence, from the earliest to the latest. Function in this sense is physiological activity of all kinds, all transformation and transference of energy.

It is a well established fact that the special functional activity of organs is very generally a factor in their development and differentiation beyond a certain stage, and in the maintenance of their characteristic form and structure after development is completed. In the absence of this functional activity the organ does not develop beyond a certain point and does not persist indefinitely. Functional hypertrophy, atrophy from disuse, functional adaptation, etc., are terms used to designate these relations between function and form.

But the real difference between earlier and later stages of development consists, it seems to me, not in the absence of function at one stage and its presence at another, but rather in the difference in character of function in the different stages. If a particular kind of function determines the structure and differentiation at one stage, and we know that it does this, is there not a logical basis for the belief that the functions which exist in other stages are also formative factors in those stages ?

In other words, is there not good reason to believe that every stage of development is directly or indirectly the necessary consequence of the functional activity in the preceding stage ? According to this view each stage of development is a machine in the broad sense and each is the product of the activity of a preexisting machine. External factors play a part, particularly in

plants and in the simpler animals, in determining the character of the machine and its activity, but this fact does not essentially alter the case.

Viewed from this standpoint, development, from its earliest stages on, is just as strictly a functional process as functional differentiation or functional hypertrophy in the stricter sense. This view does not necessitate the assumption of any special formative factors different in character from functional processes as the factors behind or underlying development. The formative factors of each stage are the functional activities of the immediately preceding stage (plus external factors). The process of development of the organism is not essentially different from the process of maintenance after development. Indeed strictly speaking, development ceases only when death occurs. The germ cell is an organism possessing a certain structure and function, and this forms the starting point. The functional activity in this structure determines the next stage of development, *i. e.*, a change in structure and therefore a change in function. This process continues and at the same time gradually approaches a condition of physiological equilibrium.

This view of development is of course no more an "explanation" than is the assumption of an entelechy or that of determinants. As a point of view, however, and as a basis for attacking the problem of morphogenesis it possesses a certain value in that it does away with various assumptions and places the problem of morphogenesis on a strictly physiological basis. To say that all organic form and structure are functional in their origin is merely to say that the problem of morphogenesis is a physiological problem and nothing more.

This is the position which I have held since the beginning of my work on regulation. I have used the word "function" with reference to any and all physiological processes and activities at any and all stages of development and have repeatedly called attention to this fact.[1]

[1] It is somewhat surprising, therefore, to find Driesch ('05, p. 790; '07, p. 180) pointing out that I have put the cart before the horse in regarding function as a determining factor in form regulation, since, as he says, an organ develops "for function" and does not function until it is developed, or at least until a certain stage of development has been reached. The difference between Driesch and myself on this

When we come to consider the process of development somewhat more in detail we find that two complex groups of internal factors must be considered, viz., constitution and correlation. This, of course, is true of any machine: its function depends upon the constitution and correlations of its parts, provided, of course, that the necessary external conditions for function are present. In different organisms and in different features of development the two factors may of course play a very different part.

2. *Functional Regulation and Form Regulation.*

It follows from what has been said that all form regulation is, according to my definition, either directly or indirectly functional regulation, *i. e.*, the physiological processess in the structure involved determine what the result of regulation shall be (Child, '06*c*).

In certain cases among the turbellaria I have been able to control, inhibit and modify the process of form regulation to a greater or less extent by controlling and modifying, in most cases indirectly, the movement and use of the parts most intimately involved in the regulatory processes (Child, '02, '03, '04*a–c*, '05*a–d*). This work showed very clearly that movement and use of parts were important factors in certain cases and certain features of regulation: they may even be primary factors in determining certain results. It is just as certain, however, that in many other cases they play no part at all.[1]

point is in reality merely one of definition; he limits the term "function" to the processes of which I spoke in the first paragraphs of this section, while I regard all processes in the organism as functions. His criticism of my position is of course entirely beside the point since he substitutes his definition for mine. Physiological processes of some kind certainly occur even in the earliest stages of development, and that these are functions of an existing structure cannot be doubted. I believe that they are also the formative factors in development.

Morgan ('07) calls attention to the fact that the functional idea is not new. This of course is true; it is the position which every physiologist must hold in one form or another. I have never considered that I was formulating a new hypothesis of development; I have merely attempted to apply certain physiological ideas to the phenomena of form regulation. Among botanists this view has been very generally held for a long time.

[1] Morgan ('07, p. 374) apparently believes that I regard movement as a universal factor in form regulation, for he calls attention to the fact that movement does not occur in many cases of regeneration. But I have repeatedly asserted that my conclusions upon the effect of movement concerned only certain species and certain features of regulation (Child, '02, p. 218, p. 229; '04*a*, p. 131; '04*b*, p. 467, etc.).

As I pointed out concerning *Leptoplana* (Child, '04*a*) movement is undoubtedly not a factor of importance in determining the formation of new tissue at a cut surface, but experimental evidence indicates clearly enough that it is a factor in determining the rapidity, amount and direction of growth and to a greater or less extent the character of its differentiation.

In the experiments on *Planaria* described above, the head and pharynx, for example, do not attain their "normal" shape and structure when movement is largely inhibited by anæsthetics. By the use of the higher concentrations it is possible to inhibit in almost any desired degree the process of form regulation so far as visible morphogenesis is concerned. Pieces which in water form heads very rapidly may be prevented entirely from forming heads by the proper use of the anæsthetic, or almost any intermediate condition between these two extremes may be attained by the formation of incomplete or partial heads. Pieces incapable of forming heads in the anæsthetic, regain their original power when returned to water. But it does not in the least follow from these facts that head formation is entirely the result of actual movement. My position is, and has been, that movement is merely one of the functional activities concerned in development, and which usually concerns later stages to a greater extent than earlier. But in regeneration in the turbellaria and in various, though by no means all, other forms a peculiar condition exists in that new tissue adjoins fully developed parts which may be in active movement. There can be no doubt that movement of these adjoining old parts influences conditions in the new tissue and so affects the result, either quantitatively or qualitatively or both. But movement and functional activity in the stricter sense, in which Driesch uses the word, are certainly far from being universal factors in either form regulation or ontogeny.

Whatever the importance of movement in a particular case, it is merely one of a great variety of functional factors. In many cases movement and motor use of parts are not concerned at all in regulation and in some other cases it is evident that while movement may affect the later stages this movement is possibly only in consequence of preceding regulation. In *Cestoplana* (Child, '05*b*), for example, where the posterior portion of an an-

terior piece becomes visibly "posterior" as regards the character of its movements before any marked structural changes occur, regulation must have occurred before such changes in movement could appear.[1]

3. *Mechanical Regulation and Morphallaxis.*[2]

In the first of the "Studies" the idea of mechanical regulation as the chief factor in the change of shape in pieces of *Stenostoma* was developed. According to my conception mechanical regulation is primarily a mechanical deformation of physically plastic tissues. Whether we consider regulation as a return or approach to the normal condition after a disturbance of this condition (Driesch, '01), or as a return or approach to a condition of physiological equilibrium after a preceding condition of equilibrium has been disturbed (my own definition), mechanical regulation in my sense

[1] In my paper I referred to these changes as functional regulation and called attention to the altered character of the movement as indicating that they had occurred, but while the movement is undoubtedly a factor in what follows, it would be absurd to suppose that it is the primary factor in such a case.

[2] Some years ago, in describing the course of regulation in *Planaria maculata*, Morgan called attention to the peculiar changes in shape which the pieces undergo, these changes consisting mainly in a decrease in width and an increase in length. Concerning this process he says: "Thus the relative proportions of the planarian are attained by a remodelling of the old tissue. I would suggest that this process of transformation be called a process of morphallaxis" (Morgan, '98, p. 385). Later ('00) he applied the same term to the changes in shape and proportion in *Hydra* and other forms. So far as I am aware, Morgan has not stated at any time whether the term "morphallaxis" is to be applied to the whole process of form regulation in *Planaria* and other forms, including the regeneration and redifferentiation which occurs, or only to the changes in shape and proportions of the piece. From the quotation given above it would appear that he intended it to apply only to the change in shape and proportions, but in his latest statement ('07, p. 15) he apparently applies the term to the whole process of form regulation by redifferentiation. In my earlier "Studies" I used the term with reference only to the changes in proportion and shape: later, however, I substituted "change in proportions" for it as less ambiguous for my purposes (Child, '05*a*, p. 253). Driesch ('01) considered the term as synonymous with "Restitution durch Umdifferenzierung," a very different meaning from that which I had given it in my work. It is probable that Driesch's misunderstanding of my conclusions concerning form regulation is in part due to our different interpretations of this term.

In several of my earlier papers the word "form" was used for "shape" and "outline." I agree with Driesch that this use of the word may be misleading, but I was careful to distinguish between form in this sense and structure, and pointed out that the factors concerned with change in form probably had nothing to do in many cases with change in structure (Child, '02, p. 218).

is theoretically possible, provided certain characteristic mechanical conditions exist in the normal animal and are altered in characteristic manner in the piece. In several of the "Studies" the changes in shape in pieces of *Stenostoma*, *Leptoplana* and *Cestoplana* were shown to be apparently primarily mechanical regulations (Child, 02, '04*a*, '05*a*, '05*c*) and it was suggested that similar processes might occur elsewhere. The recognition of mechanical regulation is not in any sense an attempt to interpret regulation in general on a mechanical basis (cf. Child, '02, p. 229): it concerns merely changes in shape and outline. Mechanical regulations are possible only under the conditions mentioned above, and whether they occur or not can only be determined by investigation of each particular case. It is not necessary to suppose that all cases of "morphallaxis" (in the sense of change in shape and proportions) are mechanical regulations. If they are they certainly depend on different mechanical conditions in different cases. In *Hydra*, for example, the factors determining the change in shape are certainly not the same as in *Planaria*, and may not be mechanical at all. In the cases which I have considered mechanical strains arise in connection with locomotion and these strains are altered in a characteristic manner in isolated pieces and, as I have shown, the changes in shape are exactly what might be expected in physically plastic material under these conditions. Mechanical regulation is to be expected only in organisms or parts possessing a considerable degree of physical plasticity and where skeletal structures are not concerned. As regards the shape of animals in general gross mechanical factors are certainly unimportant as compared with others, or not concerned at all, and I have never even suggested that such factors were of general significance (cf. Driesch, '05, p. 790). On the other hand, it seems to me absurd to deny that characteristic strains or pressures existing in plastic material may play some part in determining shape.

That the changes in shape in pieces of *Stenostoma*, *Leptoplana*, *Cestoplana*, and *Planaria* are not primarily "functional adaptations" (cf. Driesch, '05, p. 766) is, I think, evident. A functional adaptation, as I understand it, is a change in structure which involves a change in functional capacity, either an increase

or a decrease or a modification in kind according to the character of the factor inducing the change. The change in shape in the pieces of *Planaria*, etc., does not in itself involve any such change in functional capacity, moreover, functional adaptations to mechanical tension and pressure consist, usually if not always, in an altered resistance to the mechanical factor, not in a change of shape which accords with the laws of mechanics. The change in shape in these turbellaria is essentially similar to what would occur under the same conditions in any material of similar physical constitution and there is not the slightest evidence that it results from a change in functional capacity.

There is little doubt, however, that functional adaptations result from the change of shape: as was suggested above, it is probable that the cessation of the change and the final attainment of a more or less characteristic shape, *i. e.*, a condition of equilibrium, is in part the consequence of the functional reaction of the differentiating and redifferentiating tissues to the altered mechanical conditions. In other words, the change of shape brings about the functional adaptation instead of resulting from it.

But even though these changes in shape do not appear to be functional adaptations in Driesch's sense, they are functional regulations in my sense, and probably the simplest possible kind of functional regulation.

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SOME ABNORMALITIES AND REGENERATION OF PLEIOPODS IN CAMBARUS AND OTHER DECAPODA.[1]

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Introduction.

The two crayfishes whose abnormal appendages are described in this paper were found among a small number of specimens of *Cambarus virilis* Hagen, which were captured near the city of Chicago in the autumn of 1904. The discovery of these abnormal pleiopods led to an experimental study of the regeneration of the abdominal appendages in the crayfish, the results of which are given herein. The earlier experiments were unsuccessful owing to imperfect methods of handling the material, and to the use o mature animals in which the moults were infrequent. The later experiments, with improved methods of caring for the material, and with small immature specimens, have yielded satisfactory results.

These experiments were begun in the Hull Zoölogical Laboratory of the University of Chicago, continued in the Biological Laboratory of Transylvania University, and completed in the Marine Biological Laboratory of the Brooklyn Institute of Arts and Sciences at Cold Spring Harbor, Long Island. I wish to express my thanks to Dr. Davenport, director of the Marine Laboratory, who kindly placed the facilities necessary for the completion of the work at my disposal; also to Dr. A. E. Ortmann, of the Carnegie Museum, Pittsburgh, to whom I am indebted for identification of the species used in the experimental work; and to Dr. Faxon, of Harvard, and to Dr. Hans Przibram, of Vienna, for examining the abnormal specimens.

Historical Summary.

The regeneration of the abdominal appendages of decapod crustaceans has been a subject of investigation for many years, but

[1] Contributions from the Biological Laboratory of Transylvania University, No. 2.

not until recently has a critical study of it been attempted. A review of the earlier literature relating to regeneration in the Decapoda has been given by Miss Steele, '04; it is necessary, therefore, to mention only a few of the later papers upon whose results my own observations have some bearing.

Morgan, '98, desiring to test Weismann's hypothesis, that regeneration is an adaptation, experimented with *Eupagurus longicarpus* to determine what relation, if any, existed between the power of regeneration and liability to injury. Because the hermit crab lives in shells where the abdominal appendages are protected from injury, whereas the thoracic appendages are not so protected, these two series of organs were chosen for this experiment. He came to the general conclusion that no such relation existed, and further supported this view by a second paper in 1900. The latter paper was based upon experiments in which the thoracic appendages were removed at unusual levels.

He found in the course of his experiments that the abdominal appendages of *Eupagurus* did not regenerate readily, although slight regenerative power existed. He suggested that this rarity of regeneration "may be connected in some way with the amount of food supply brought to the region from which they arise."

Miss Steele, '04, experimenting with *Cambarus virilis* and *C. gracilis* has obtained results similar to Morgan's. She says, speaking of the swimmerets: "I have found none to regenerate except the first pair in the male. . . . In the case of the other abdominal appendages except the sixth pair, regeneration, if it does take place, is very slow in beginning."

Emmel, '04, reported the observation of regeneration in the first appendages of the lobster. He experimented with the other appendages and says: "In experiments with the other four pairs of abdominal appendages or swimmerets, positive results were obtained in the second and third pairs, and it seems safe to say that all the swimmerets will regenerate."

Haseman, '07, mentions the regeneration of swimmerets in *C. propinquus*, giving figures showing the progress of differentiation, but he does not mention the fact that the regeneration of these appendages was considered doubtful.

A discussion of the results obtained by Morgan and Miss Steele will be found in connection with the discussion of my own observations in a later portion of this paper.

Abnormal Swimmerets.

Anomalous variations not of congenital origin are of little interest to the student of evolution; for it has never been definitely demonstrated that any acquired somatic variation is inherited. Moreover, no very important laws of variation are likely to be discovered by a study of teratological specimens. Nevertheless, it is advisable to place interesting cases of abnormal growth and regeneration on record, for we may finally acquire a knowledge of the physiology and mechanics of development sufficient at least to explain the origin of such anomalous variations.

The swimmeret shown in Fig. 1 is the right appendage of the second abdominal somite of a female *C. virilis*. The pleuron is much deformed, as is readily seen if compared with the normal pleuron of the opposite side of the same somite. The coxopodite of the abnormal appendage is much larger than normal. From this enlarged basal piece arise two appendages, one of which, near the posterior side of the coxopod, seems on casual observation to be a beautiful case of duplicity. The parts are perfectly doubled from the proximal portion of the basipodite outward. The two pleiopods thus united are normal in every particular beyond the point of union, the posterior member being somewhat larger than the anterior. From the anterior portion of the large coxopodite, completely separated in point of origin from the double pleiopod, arises another appendage which is uniramous, but of a size and structure typical of the endopodite of the second pleiopod.

It is an interesting circumstance that the first pleiopod lying immediately anterior to the one just described is also abnormal. This swimmeret is shown in Fig. 2. The lower portion of the appendage is much enlarged, and at the proximal end of the basipodite, there is a posterior projection set with stiff hairs, reminding one somewhat of the structure of the coxopodite of the pereiopods.

The third abnormal swimmeret is from a male *C. virilis* which

was found to have three pairs of appendages instead of two, modified for sexual purposes. The right swimmeret of the pair is shown in Fig. 3, the left one being exactly like it. The character of the modification in this third pair is just the same as that of the second, except that the projection of the endopodite is smaller than on the second. The first and second pairs of appendages are perfectly normal. Moenkhaus, '03, has reported an exactly similar case in the same species, and so far as I know these are the only two cases on record.

The Problem, Materials, and Methods.

Being convinced that the abnormal swimmerets of the female *C. virilis* described herein were the result of regeneration after a somewhat extensive injury, and having before me the work of Morgan on *Eupagurus longicarpus*, and of Miss Steele on *C. virilis* and *C. gracilis*, I determined to perform a series of experiments with the crayfish in order to ascertain whether or not the abdominal swimmerets regenerate, and if so, under what conditions. These experiments were begun in 1905; and after two years of rather unsuccessful work the choice of material and the methods of handling it were so improved that gratifying results have been obtained.

At first attempts were made to keep the crayfishes in aquaria of running water. A large aquarium was divided by partitions of galvanized iron netting into a number of compartments, each about 30 cm. square. Each compartment was provided with gravel and flat stones, and fresh water from Lake Michigan was kept running constantly at a depth of about 5 cm.

The aquarium was cleaned frequently, and all precautions were taken to keep the animals in sanitary conditions; but in spite of the great care exercised, the crayfishes would die after several weeks of confinement. Since my first material was adult, and moulted therefore infrequently, death took place before any moults occurred. Many variations of these conditions were tried in an attempt to secure more favorable results, but without success.

During the last two years a method has been employed, which has obviated all difficulties, and has given entirely satisfactory

results. Young specimens of *Cambarus* (*Bartonius*) *bartoni* Fabr., probably at the beginning of their second and third years were taken on March 24, 1907, from a small stream which crosses the Bryan Station pike about three fourths of a mile northeast from the city limits of Lexington, Ky. The specimens used are not like the typical *C. bartoni* of the eastern United States, but have a narrower areola, less spiny carpus, and a shorter but broader rostrum than the eastern form. Dr. Ortmann states that they agree with specimens described by W. P. Hay in the 20th Ann. Report of the Ind. Geol. Survey, 1895, after comparing the living specimens with those in the Carnegie Museum from Mitchell, Lawrence Co., Ind.

Each specimen was put into a tumbler with water not quite sufficient in amount to cover it. A small piece of mica schist coming not quite to the surface of the water, was placed in each tumbler so that the crayfishes could crawl upon it and expose themselves to the air at will. The tumblers were kept nearly covered by glass plates to prevent accidental desiccation. Care was taken not to cover the dishes entirely, as the air was found to become overcharged with carbon dioxide in a short time if so covered. The water was changed completely three or four times per day, and even oftener on very hot days; for crayfishes seem unable to endure warm water for any great length of time.

The water used in these experiments was supplied by the city water works of Lexington. This water is filtered through large Jewell filters before being pumped into the water mains, and is exceptionally pure. The complete change of such water every few hours rendered the multiplication of bacteria or growth of other fungous plants rather difficult, and tended to prevent the accumulation of any sediment.

When it appeared that any fungous growth was forming upon the appendages and about the thoracic region, each crayfish was treated for a few minutes with a bath of copper sulphate. The vessels and stones kept in them were occasionally treated with the same solution. The copper sulphate was used with a strength of one part to a million, when the animals could be left in it for some time. Solutions much stronger than this, one part in ten thousand, can be used for a few minutes at a time with entire suc-

cess in destroying bacteria and fungi, and without injury to the crayfishes, provided they are washed thoroughly several times in plenty of pure water after the bath to cleanse their gills of the copper sulphate.

Young crayfishes are voracious creatures, and need to be fed frequently. I have fed them every day with entire success, but they soon tire of a uniform diet. Several kinds of food were used; and by rotation, so that they never received the same kind of food on successive days, their appetites were retained. Fresh raw beef was given them twice a week, and raw potatoes and pieces of *Myriophyllum*, which was kept growing in the laboratory, were used as vegetable foods. The *Myriophyllum* was covered with slime, which was found to contain unicellular algæ, rotifers, nematode worms, annelids, such as *Æolosoma hemprichii*, and other kinds of small animals. This slimy material was especially esteemed, and probably most nearly represents their normal diet. This food material was left in the glass with the animals for an hour or two, after which the remains were removed and fresh water placed in the vessels.

Using these methods, I have kept them alive for months in perfect health, with rapid growth during the moulting season. Occasionally a death would occur among them, but these fatalities were always obviously due to special causes, not to any general defects in the methods employed.

After ecdysis the cast off exoskeletons were used in the preparation of the drawings.

Experiments.

The experiments here described are a few typical ones selected from a series, all of which gave similar results. The numbers correspond to those used while recording notes on individual experiments.

No. 4. *C.* (*Bartonius*) *bartoni*, ♀. Fifth right abdominal appendage removed March 24, 1907. The appendage was cut off, leaving a short stump attached to the body (Fig. 4).

The first moult occurred in the afternoon of March 27, three days after the operation. No regeneration could be noticed, but the wound was perfectly healed (Fig. 5). The second moult

occurred during the night following May 6, 1907. Between March 27 and May 6 no change could be detected even by use of the microscope. But that changes were taking place beneath the chitinous cuticle is evident from the expansion of the regenerating limb, which occurred immediately after this second ecdysis (see Fig. 6).

A third moult took place on June 19, 1907, at which time the appendage again expanded, reaching the size and condition shown in Fig. 7. The regenerated appendage measured at this time 3.3 mm. in length; the one on the opposite side of the same somite 4.8 mm. (Fig. 8). The regeneration was equal to nearly 69 per cent. of the normal size from March 24 to June 19, a rate which I consider by no means slow.

Owing to an unfortunate accident this crayfish was killed on the morning of July 5, 1907. Had it lived until after its fourth moult it would probably have shown a much more nearly complete regeneration of the appendage.

No. 7. *C.* (*Bartonius*) *bartoni*, ♀. Fourth left pleiopod removed March 24. The first moult after the operation took place the night of April 22, 1907. The amount of regeneration while not extensive was plainly visible. The second moult occurred May 25, 1907. The regenerating appendage expanded to nearly half the natural size, immediately after the exoskeleton was cast (Fig. 9).

No. 3. The same as No. 7 except that the fourth pleiopod was removed from the right side instead of the left. The first moult, on March 30, showed a small whitish papilla a little over one mm. in length, projecting from the base of the amputated limb. This projection increased slightly with age, but this individual was killed in July by the water in the vessel receiving direct sun-light through a partially open window blind, thus becoming much overheated. Fig. 10 reveals internal development. A later moult would probably have given results similar to No. 7. This specimen was probably a year, or perhaps even two years, older than No. 7, and consequently only one moult was recorded between March 24 and July 5, while some of the smaller, younger specimens moulted three times in a shorter period (cf. No. 4).

No. 2. *C.* (*Bartonius*) *bartoni*, ♂. The third right appendage was cut off March 24. On May 9, no moult having occurred, the stump of the appendage was cut longitudinally by means of sterilized scissors. The moult occurred on June 17, but no abnormal growth was noted. The appendage was perfect, and showed regeneration of more than 50 per cent. in size (Fig. 11).

No. 5. *C.* (*Bartonius*) *bartoni*, ♀. The second left appendage was removed March 24, 1907. The first moult occurred on May 8, 1907, and the regeneration amounted to about 30 per cent. The second moult took place on June 26, 1907, when the appendage showed about 65 per cent. of complete regeneration. Fig. 12 shows the point of amputation, and Fig. 13 the appendage after the second moult.

No. 6. *C.* (*Bartonius*) *bartoni*, ♂. Sixth right appendage was amputated just beyond the basal joint March 27, 1907, as shown in Fig. 14. It moulted the following day. A very narrow edge of white tissue was visible along the cut edges, but it is not probable that any regeneration had taken place. During the time preceding the next moult which occurred on May 27, the basal portions of the rami increased somewhat in size (Fig. 15), and after moulting the appendage was about one half natural size, and perfectly formed (Fig. 16).

A series of experiments on the pleiopods of *Palæmonetes vulgaris* was carried on at Cold Spring Harbor, and regeneration of all the abdominal swimmerets takes place rapidly in young specimens.

At present a series of experiments to test the regeneration of the antennæ from various levels and to compare the rate of regeneration of pleiopods and pereiopods in *C.* (*Bartonius*) *bartoni* is being carried on; but the data are insufficient as yet to permit a general statement regarding either phase of the series.

Discussion.

A. *The Abnormalities:* — As far as I have been able to ascertain, only one abnormal abdominal appendage has been recorded for any of the decapod crustaceans. In as much as all abnormal pleiopods described herein or elsewhere have been discovered accidentally, the rarity of such records is due in part, perhaps, to

lack of careful observation; but the examinations which have been made show that they occur infrequently.

The abnormalities presented here are of interest for several reasons. The abnormal pleiopod is evidently the result of a regeneration after extensive injury in one instance, and just as evidently the result of undisturbed growth processes in the other instance. That is, the condition is congenital and may be considered as having arisen by mutation.

It would appear that the swimmerets shown in Figs. 1 and 2 probably resulted from an injury which removed part of the pleuron, and tore the first and second pleiopods near the base of each, as represented diagrammatically in Fig. 17. The coxopodite in Fig. 1 is abnormally large, which condition may possibly be explained by the fact that a large area was exposed when the mutilation occurred.

Very few genuine cases of duplicity have been described. Bateson mentions only four cases, all chelæ; Herrick has figured a double chela of a female lobster, and Zeleny has described a double chela of *Gelasimus pugilator* which regenerated instead of a normal single one during the course of his experiments.

Bateson states emphatically that "in arthropods and vertebrates such a phenomenon as the representation of one of the appendages by two identical appendages standing in succession is unknown. No right arm is ever succeeded on the same side of the body by another arm properly formed as right, and no crustacean has two right legs in succession where one should be."

While this supernumerary appendage may be regarded as a complementary image of the normal one, and therefore a left appendage instead of a right, there is nothing in the structure of either member to indicate that such a relation exists. The members are not imperfect, and are placed in succession, differing in these two respects from the cases admitted by Bateson.

At first appearance the coxopod of the abnormal first appendage is very much like that of the last pereiopod. And the anterior uniramous part of the second pleiopod is like the first pleiopod in being uniramous, but is a typical endopodite. There is a mere suggestion here of a shifting backward of the series of organs, a condition to which Bateson applied the term backward homœosis.

However if the injury actually took place as indicated in the diagram referred to above, the most plausible explanation which can be offered is in harmony with Bateson's statement. The regeneration should lead theoretically to triplication, that is, to the production of three appendages, two supernumerary ones on each cut appendage; and these on the right side of the body should stand as a left between two rights. The abnormal pleiopod shows defective regeneration only in the suppression of the exopodite of the anterior supernumerary appendage. The rounded projection of the first accessory pleiopod may be explained similarly as the fused basal portions of the two supernumeraries, whose tapering jointed ends have been completely suppressed.

The abnormalities have probably arisen after an injury caused by some force acting in the direction of the arrow in the diagram, Fig. 17, which produced two breaking surfaces, from which the new pleiopods arose, following the laws of symmetry for supernumerary appendages as stated by Bateson.

The abnormal appendage shown in Fig. 3, is a case of backward homœosis. It would be of interest to know the hereditary behavior of such unisexual characters if bred in confinement.

B. *Experiments.* — Few investigations of the regenerative power of the abdominal appendages of the Decapoda have been made, and the results obtained have not been entirely satisfactory. Morgan's experiments on *Eupagurus longicarpus* in 1898 showed that a slight power of regeneration existed in the appendages of two or three of the individuals he used; but his experiments extended over too brief a period of time to secure any marked regeneration. The first experiment was continued only twenty days, and the second for twenty-eight days. And the conditions under which the material was kept were possibly not the most sanitary, as the fact that over 40 per cent. of the individuals died during the twenty-eight days would seem to indicate. One would hardly expect regeneration to be rapid under conditions in which life itself could scarcely be maintained. My experience has shown that although regeneration may occur after an operation, and become visible without an intervening moult, it usually does not do so in the pleiopods. None of the individuals Morgan used was kept until a moult had occurred.

Miss Steele's experiments on *C. virilis* and *gracilis* were carried on for a sufficient length of time, but she found it difficult to provide the sanitary conditions necessary to such prolonged experimentation. The specimens used in her work were probably too old to give the best results. They measured not more than three inches in length, a size which would indicate that they were several years old at least. The younger the specimens used, the more frequently the moults take place. Those used in my experiments measured from one and six tenths to two and five tenths cm. in length, and were probably at the beginning of the second or third year. Two individuals measuring five cm. in length were also used. These did not yield as satisfactory results although some regeneration occurred. The difference was probably more in the frequency of moulting than in anything else. While one moult took place in these older ones, I could secure three in the younger. And to have kept the older individuals until the same number of moults occurred as in the younger ones, would have required two years instead of four or five months. The use of more nearly adult material, and the fact that slight attention was paid to the swimmerets may account for the slight regenerative power which Miss Steele was able to report.

My experiments show that the swimmerets of young specimens of *C.* (*Bartonius*) *bartoni* regenerate rapidly, and that the regeneration of any of the appendages may be practically completed in a single season of growth. The regeneration is not particularly slow in beginning, having become visible in one instance only six days after the operation was performed. It is usual, however, to find that the regeneration begins and takes place under the old exoskeleton, without showing any visible indications that the new parts are forming. For instance, No. 9 moulted on March 28, four days after the operation, and no regeneration could be noticed. The next moult took place April 30, but during that time no indication whatever that regeneration was occurring could be seen. Nevertheless, when the moult occurred it was evident that regeneration had taken place. If the experiment had been continued for only thirty days, the conclusion naturally drawn would have been that regeneration either did not occur, or was " very slow in begin-

ning." As a matter of fact there is no way to tell how soon after March 28 the regeneration did begin, if indeed it had not already begun at that time.

To explain the slight regenerative power which he found in the abdominal appendages of *Eupagurus*, Morgan suggested that the food supply of these organs might be considerably less than that of the thoracic legs. It seems to me quite unnecessary to make this assumption, especially since I have shown that there is a rapid and complete regeneration of the swimmerets in young specimens of *C.* (*Bartonius*) *bartoni*. In this connection Emmel, '04, observed that swimmerets in the lobster will regenerate more rapidly than the pereiopods if the latter are cut "only a relatively short distance below the breaking plane." And he questions whether the supply of food material can explain the comparative difference in the regeneration of pereiopods and swimmerets. Moreover, if the limited food supply is responsible for lack of regenerative power how must we regard the regeneration of two supernumerary appendages in the abnormal swimmeret figured? It seems to me that some other explanation must be offered for the difference in regenerative power.

It has been a common experience with those who experiment with regenerative tissues, that the regeneration is always more rapid and complete in young individuals than in old ones. This fact is due probably to the greater plasticity, the more active and mobile condition of young tissues. They are more nearly embryonic in character, differentiation is not so complete, nor so fixed as in the older tissues. Emmel's ('08) recent work on the reversal of asymmetry in the lobster lends emphasis to this statement. He says: "In the first four stages of the lobster's development, a crusher may be produced on either the right or the left side of the body by the autotomous amputation of the chela on the opposite side — the regenerated chela becoming a nipper. During the fifth stage, although the chelæ are still symmetrical, the possibility for such experimental control disappears."

Since differentiation is known to proceed at different rates in different parts of the body, we may suppose that one part retains its primitive condition longer than another. If any portion of the adult regenerates less rapidly than another portion, may

it not be an index of the comparative plasticity of the two parts? The more rapid regeneration of the thoracic appendages than of the swimmerets in the adult crayfish, may be due to a longer retention of the plastic embryonic condition of the cells in the region of the breaking joint by the former, certainly not to any proportionate difference in the food supply of the two series of organs.

The important point in Morgan's results which he properly emphasized, was that regeneration did take place, however slight it might be. While we cannot say that the abdominal appendages of crayfishes are never injured, yet injuries to them are rare in nature. Examination of hundreds of individuals has shown that there is a remarkable uniformity of size and structure in these organs, a condition which could not exist if mutilations were frequent. Yet these appendages possess as high a power of regeneration in youthful stages as any of the appendages which are so frequently torn away. My experiments strengthen very much the evidence in favor of Morgan's statement that there is no relation between power of regeneration and liability to injury.

Conclusions.

The following conclusions may be drawn from the study of abnormalities, and the results of the experiments described in this paper.

1. The abdominal swimmerets of *C.* (*Bartonius*) *bartoni* Fabr. all possess a high power of regeneration in immature specimens. Since regeneration of swimmerets has been noted in *Cambarus propinquus*, *Palæmonetes vulgarus*, *Homarus americanus* and *Eupagurus longicarpus*, it is probable that the decapods generally possess this power, especially in young individuals.

2. This regeneration usually cannot be seen till after one, and sometimes two, moults have occurred, due to masking of the regeneration by the exoskeleton.

3. Slow regenerative processes in the swimmerets of the older individuals are due probably to a lower degree of plasticity in the protoplasm rather than to insufficiency of the food supply.

4. Injuries may occur, but they are rare in swimmerets, and the power of regeneration and liability of the parts to injury are apparently independent.

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Explanation of Plate I.

All figures except Fig. 17 are $\times 5$.

Fig. 1. Second right pleiopod of *C. virilis* Hagen, ♀, showing two supernumerary appendages.

Fig. 2. First right pleiopod of the same specimen.

Fig. 3. Third right pleiopod of *C. virilis*, ♂, modified for sexual purposes.

Figs. 4–7. Successive steps in the regeneration of the fifth right pleiopod in *C.* (*Bartonius*) *bartoni*, ♀.

Fig. 8. Left pleiopod from same somite as Fig. 7, without regeneration, drawn to same scale.

Fig. 9. Fourth left pleiopod of young *C.* (*Bartonius*) *bartoni*, ♀, after two moults.

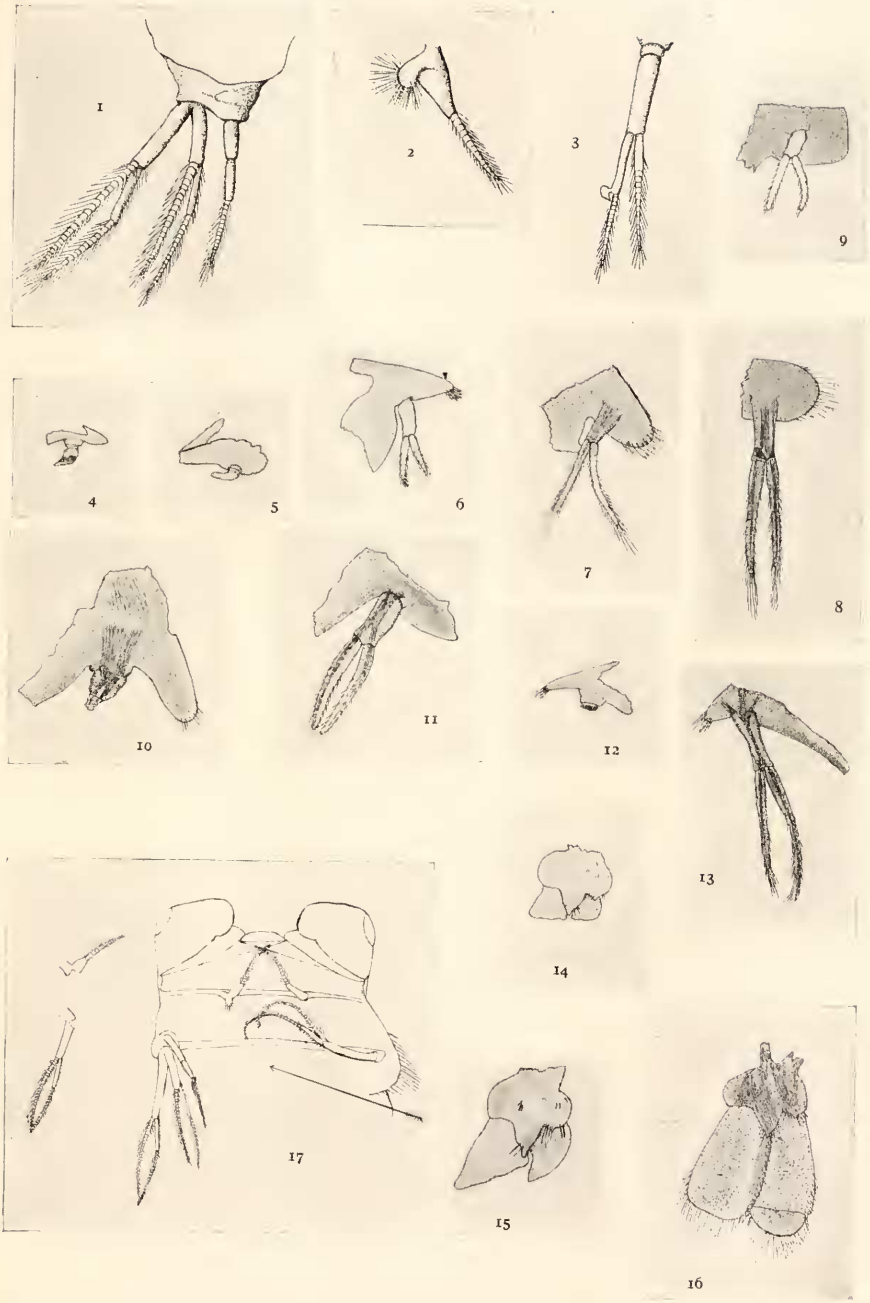
Fig. 10. Fourth left pleiopod of older *C.* (*Bartonius*) *bartoni*, ♀, after three and one half months.

Fig. 11. Third right pleiopod of *C.* (*Bartonius*) *bartoni*, ♂, after one moult.

Figs. 12–13. Second left pleiopod of *C.* (*Bartonius*) *bartoni*, ♀, condition immediately after amputation, and after the second moult.

Figs. 14–16. Right uropod of *C.* (*Bartonius*) *bartoni*, ♂, condition at amputation, before, and after, second ecdysis.

Fig. 17. Diagram illustrating the way in which the abnormal appendages shown in Figs. 1 and 2 may have been produced.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SEX RECOGNITION IN CYCLOPS.

S. J. HOLMES.

The sexual behavior of copepods presents several points of similarity with that of the amphipods which was described by the writer in a previous paper.[1] In both groups the males clasp and swim about with the females for a long time previous to copulation ; and in both groups the behavior of the female is much the same while being clasped by the male. Having an opportunity to study a thriving culture of *Cyclops fimbriatus* in which pairing was actively going on the endeavor was made to ascertain if the method of the sex recognition employed in the amphipods occurs also in this species of a quite distantly related group.

Male *Cyclops*, as is well known, have the first antennæ enlarged and modified to form a clasping organ. In *Cyclops fimbriatus* the male usually clasps the female just in front of an enlargement at the base of the abdomen. Females carrying eggs are sometimes seized, and also females not more than half grown. Males show great eagerness in grasping the females, and they can be compelled to release their hold only with difficulty. They may be poked about roughly with a needle and the posterior part of the body may be cut off without causing them to leave the female. I have often picked up pairs in a fine pipette and forcibly squirted them out several times without succeeding in separating the two sexes.

As the pairs of *Cyclops* swim through the water the males are usually the more active. Frequently the female remains entirely quiet with the appendages drawn close to the body, and the body flexed ventrally, allowing herself to be passively carried about by her mate. At other times the female may swim as actively as the male. In general the behavior of the females and their attitude while being carried closely resemble what is found among the Amphipoda. So also does their behavior when the males come in contact with them and attempt to seize them. The

[1] Biological Bulletin, Vol. 5, 1903.

female during the efforts of the male to clasp her around the base of the abdomen usually lies quiet with the appendages drawn close to the body. She may be seized by the legs, tip of the abdomen, or any other part of the body, but the male works around until he gets into his normal position which he sometimes attains only after much labor. Females vary greatly however in respect to their willingness to be clasped by the male, certain individuals resisting seizure for a long time.

So far as could be detected the males do not seek or follow the females at a distance as Parker concluded they did in *Labidocera*. The association of the sexes seems to be due merely to chance collisions. Males often attempt to seize other copepods with which they collide regardless of their sex. The males resist such attempts at seizure and dart quickly away, while the females often stop and submit readily to the clasping propensities of their companions. Several males were injured so that they could not resist seizure, and in many cases they were seized by other males who worked industriously until they got their burden clasped around the base of the abdomen in the usual way. These associations did not last long however; the active males apparently appreciating that something was wrong soon swam away. Recently killed females were often seized and in some cases carried about for a while, but they were finally dropped. Males seem rather more prone to seize dead females than members of their own sex. In one case I saw three males tugging away at a dead female, and they were soon joined by a fourth male who participated in the same effort.

It is possible that the odor of the female determines to a certain extent the sexual behavior of the males, but my experiments yielded no evidence of this. Several females were put into a tube one end of which was covered with fine gauze and the tube was then placed obliquely in water in which were numerous males. The males showed no tendency to congregate around the end of the tube where the females were confined. In another experiment several females were placed in a glass tube in which a small plug of loose cotton was inserted a short distance from one end. This end was laid obliquely in the water. The males showed no tendency to enter the open mouth of the tube as they

might be expected to do if they were attracted by the odor of the females. The experiment of removing the organ of smell which was performed in the case of the amphipods would be a fruitless one in *Cyclops*, as the seat of smell is located to a considerable degree at least in all probability in the same organs that are used for clasping.

It is evident that mating in *Cyclops* is brought about much as it is in the Amphipoda. The males have a strong tendency to clasp other copepods; the females tend to remain quiet in a condition somewhat resembling the death feint while being seized by the males. It is not improbable that olfactory stimuli may cause the males to remain with the females longer than they otherwise would, and they may render the males rather more prone to seize females than other males, but so far as could be determined by watching the behavior of the animals the specific reaction of the two sexes to certain kinds of contact stimuli is the main factor in bringing about their association.

OUR KNOWLEDGE OF MELANIN COLOR FORMATION AND ITS BEARING ON THE MENDELIAN DESCRIPTION OF HEREDITY.[1]

OSCAR RIDDLE.

Hardly a year has passed since the rediscovery of Mendel's Law without several additions to its descriptive terminology. This may signify either one of two things: a very healthy and vigorous growth, or the onset of senescence. Some of these newly introduced features are plainly justifiable; but there is reason to believe that the rather long series of extensions which has been made in recent times carries as its result not so much description of fact, as of deduction and far-reaching theory.

The facts and phenomena discovered by Mendel, and the array of facts of high importance which later workers in this field have brought before biologists, have already proved their value. The proved value of these facts is, however, no proof of the correctness of Mendelian interpretations of the processes of inheritance. I shall here present some *facts* which seem to indicate that these Mendelian *interpretations* are not sound; and further, that these unsound interpretations now stand as a formidable block in the path of progress to a better knowledge of the mechanism of inheritance and development.

Mendelian workers think that they have discovered, and certainly they have named and labelled, many "factors"[2] as necessary for the production of some single characters. These workers tie *all* of these factors together, and — for them — together they go into the germ cells, and whatever appears — or fails to appear — in the zygote is interpreted in terms of the

[1] Read January 19 before the Biological Club of the University of Chicago.

[2] The word *factor* as used here in a purely Mendelian sense represents quite a different thing from the physiological sense of the same word. The whole series of ordinary environmental factors — temperature, light, reaction of medium, concentration, moisture, etc., all these would not constitute even *one* Mendelian "factor." The Mendelian "factor" is often a rather unidentifiable thing, but it is conceived of as something capable of residence in, and of segregation by, the germ cells.

presence or absence (*dominance*, *inhibition*, *contamination*, etc., made use of by some workers) of *particular factors in the gamete*. But facts at hand, despite an opposite contention, will go very far towards showing that the method of analysis of the Mendelian worker has not permitted him to decide the question as to the *real number* and *separateness* of the factors; nor yet to determine as to whether certain of the factors were at all *represented in the germ cells*, or whether they may not have arisen during the ontogeny as a direct result of tissue differentiations, through regulatory processes, or otherwise, and quite independently of the existence of a definite determiner in the gamete or germ cells.

As Mendelism has developed, it has lent support to the doctrines of preformation, unit characters, and discontinuous variation. The facts and interpretations here brought forward disclose, on the other hand, no small amount of epigenesis, and strongly support the proposition that present and new knowledge will lessen, not widen, the apparent gap between discontinuous and continuous variability. There is, too, at present a marked tendency in some quarters to further elaborate and extend the "factor" hypothesis, which furnishes an additional and specific reason for my calling attention to some facts from my province of study which indicate that already we have represented too many factors in the germ cells; that qnite certainly some factors which have by Mendelian interpretation been made to circulate through the germ cells are never represented (in the Mendelian sense) in these cells at all; and finally that many factors considered most separate and discreet by Mendelians, can now be proved to be but points in lines of perfect continuity.

It will no doubt be urged by some Mendelians that the observations recorded here are quite wide of the mark because the writer has no experience in animal breeding. It is very true that I have not personally carried through any breeding experiments whatever. For information in this field I have depended upon what I have been able to see of the breeding and hybridization experiments conducted by others, and upon the literature of the subject. My own work for several years has been largely in the field of developmental and color physiology; its aim being to get

at the basis of the color characters of organisms. It must rest with biologists generally, however, to decide whether the facts here presented have, or have not, to do with the Mendelian interpretation and description of the processes involved in heredity and development.

The basis, then, of my objections to much of the Mendelian interpretation rests upon chemical and physiological facts regarding the origin and development of melanin pigments. It is necessary to anticipate the query as to how, or by what right, has melanin color formation anything to do with the essential points of Mendelism? I realize fully that the line of contact between these two provinces of activity is apparently not a line of contact at all, and so new and untrammeled is the territory that one would almost hesitate to enter, had not a pair of such good Mendelians as Cuénot and Bateson already knocked importuningly at the gateway which leads into it.

It should be recognized at the outset that, in thus presenting a body of facts from one field, as having important bearing on facts and theoretical deductions in another field, there is every risk that a short presentation will be incomplete, inaccurate, and at the same time may fail to properly or sufficiently orient the reader with respect to the writer's point of view. It is here impossible entirely to avoid incompleteness, inaccuracy, and but partial explanation of an opposed interpretation of the facts of color development and inheritance; it is hoped, however, that a presentation, and rather general though cursory discussion, of a limited number of facts — facts with which most biologists are not familiar, and which have never before been treated in this connection — will make it possible to recognize that some points of present biological theory are involved.

And, though many of my statements concerning such points of theory may seem dogmatic, I should like to make it clear that I am not deceived or blind to the fact that my present function is merely to introduce subject matter for a chapter, not to conclude a volume; to propose, not to decide. These discussions of theory would have been omitted entirely from this paper if it had been thought that the facts here brought for the first time into the field of heredity and developmental physiology would

receive the attention which they deserve without such a setting. I am, of course, rather confident of the correctness of the point of view set forth. I am absolutely convinced that the facts here presented will prove valuable assets to the student of development and inheritance.

There are three reasons why melanin color formation, better than any other process or group of processes, may furnish the starting point for certain inquiries and criticisms regarding the way Mendelian inheritance is construed and described:

1. Color characters have been more extensively studied and described from the Mendelian standpoint than have any others. A very considerable share of the color investigated — all mammalian color, for example — is due to melanin pigment.

2. It was to recognize a fact in melanin color development that Cuénot ('03) introduced the idea of *presence* and *absence* of a character, or character determiner, etc.; an idea which is now made by many workers to support practically the whole structure of Mendelian description and interpretation. Again, the now rather elaborate terminology introduced by Castle is based almost wholly upon the behavior of melanin colors. A few paragraphs of the paper by Cuénot furnish practically all there is of a tangible basis for representing *chromogens*, *enzymes*, and *activators* in gametic formulæ.

3. There is already at hand a certain amount of definite chemical knowledge, and some reasonably safe physiological information, which can be brought to bear on some points of the color philosophy of Mendelism. There is, moreover, something which though apparently less substantial, is none the less important — namely, the assurance of further, definite light from these same sources. There can be no doubt that we can use biochemical and physiological methods and data to give us what is now more needed than all else, perhaps, in the study of evolution and development — namely, *the intimate developmental history, and nature of some one character;* I mean the *proximate* history, the mechanics of what some would call the "late stages" of the development, or the "differentiation" of a character.

It may help to keep the reader oriented throughout this dis-

cussion to state that I shall first describe some of the facts of color development as they are known at present from chemical, pathological, and physiological experience ; and afterward sketch very briefly the nature of the Mendelian terminology ; this to be followed by some discussion of my point of view. Such facts of color will be considered as have bearing on the following points :

Do the known facts of the *genesis*, *nature* and *history* of color characters harmonize with, supplement, modify, or radically differ from, the demands of present Mendelian interpretation? Do they enable us to decide as to whether color characters are qualitative or quantitative in nature? Are color differences cases of continuous or discontinuous variability? Can these facts throw light upon the existence or nature of unit characters? What of the purity of gametes? Do these facts indicate a different or sounder basis for the interpretation of Mendelian, or other inheritance? What justification or light, if any, is thrown upon the present practices of (*a*) adding "factors" in order to account for the inheritance phenomena exhibited by a character ; (*b*) of tying all these "factors" together and postulating that all pass (by means of their representatives) through the germ cells?

Some Facts of Melanin Color Formation.

In a consideration of the facts of the origin of melanin coloration, one might deal at some length with the *distribution* and *histogenesis* of melanin. Though several interesting and illuminating facts lie in each of these directions, I shall dismiss these two phases of the origin of melanin colors with the single statement that the melanins are usually dark, amorphous or granular pigments, chiefly of intracellular, animal origin ; extending within this kingdom from the trypanosomes (Protozoa) to man. There is no vertebrate species (unless we may think of pure albinos as such), but has this coloring matter in one or several parts of its body. It is, however, the chemical and physiological phases of the origin of color that it is most desirable to discuss, and it is from this angle of approach that we find most of the facts which bear on the Mendelian description of heredity.

Our knowledge of what has been called the "mechanics of

melanogenesis" may be thought of as having begun with studies in the production of artificial melanins, and the accompanying search for the (melanin) chromogen in the albumen molecule. This work was shared by many workers: Stadelmann ('90), Gmelin ('94), Nencki ('95), Schmiedeberg ('97), Chittenden and Albro ('99), Hofmeister (see v. Fürth, '04), v. Fürth ('99, '01, '04), Hopkins and Cole ('01, '03), Schneider ('01), Samuely ('02) and others. Through these workers it was made known, first, that melanins artificially produced are essentially the equivalents of natural melanins; and second, that tyrosin and related aromatic compounds are the chromogens concerned.

The second step in the progress of this knowledge was concerned with the nature of the process by which the melanin is formed from the chromogen. Hlasiwetz and Habermann ('73) had first recognized oxidative processes as necessary for the formation of the artificial melanins. Landolt ('99) extended this fact to the natural pigment of the choroid.

Bertrand then discovered ('96) an oxidizing enzyme — tyrosinase — which was able to transform tyrosin into melanin-like bodies. Bertrand found the enzyme in certain plants. It has since been found to be of wide distribution, having been found by Biedermann ('98) in the contents of the alimentary canal of meal worms; by Lepinois ('99) and Gessard ('01) in the adrenal glands; by Gonnermann ('00) in beet roots; by v. Fürth and Schneider ('01) in the hæmolymph of insects; by Przibram (see preceding, '01) in the ink-sacs of cephalopods (*Sepia*); by Ducceschi ('01) in the blood of *Bombyx;* by Gessard ('02*a*, '03*a*, '03*b*) in the ink-sacs of *Sepia*, in the integuments of insects, and in melanotic tumors of horses; by Dewitz ('02) in the blood of certain insects; by Durham ('04) in the skins of mammals and birds; by Weindl ('07) in the skin, eyes, ink-sacs and eggs of *Loligo;* and by Bertrand and Mutermilch ('07) in wheat bran. v. Fürth and Schneider ('01) concluded that "tyrosinase-like ferments are widely distributed in the animal organism, and probably always appear wherever and whenever a physiological or pathological formation of melanin occurs."

Meanwhile, another advance in our knowledge of melanogenesis was made when Dewitz ('02) demonstrated the rôle of an

oxidizing enzyme (tyrosinase) in the normal development of the dark pigment of the integuments of living, growing animals (fly-larvæ — *Lucilia Cæsar*). At the same time he was able to prove that, in the forms with which he worked, free oxygen is also an indispensable factor in the development of the color. This work, important and suggestive as it was then, is now made still more valuable by new knowledge of the chromogen — that is, the other factor involved in the pigment formation. Without knowing just what this chromogen might be, Dewitz was able to conclude (p. 45), "We cannot doubt that we have here in the blood of the larvæ an enzyme under the influence of which a chromogen is oxidized and forms a brown or black pigment."

A year later Gessard ('03*b*) was able to show that in the melanotic tumors of white horses not only tyrosinase but free tyrosin is present. He concludes (p. 1088): "Tyrosin is then the chromogen, the oxidation of which by tyrosinase determines the formation of the black pigment which is common to many physiological and pathological products of the animal economy; and it can be said that the color of the negro is due to the same reaction that produces the ink of the squid, or the black color of some mushrooms." Gessard states, too, that when tyrosin is oxidized with tyrosinase it gives a series of colors — "rose, rouge-grenat et brune." In a later work ('03*d*) he made a closer study of the color reactions of tyrosin in which he showed that the presence of acids, alkalis and salts have marked effects on the colors produced.

The recent work (May, 1908) of Bertrand, is, however, of the highest interest. He has been able to determine (1) the *type of substance — of which there are many representatives — which can by the use of tyrosinase be oxidized to melanin compounds;* (2) *he has shown that each one of these compounds passes through a series of colors before arriving at the final stage of oxidation*; (3) *that this series varies somewhat as to the exact tint of the initial and final colors*, but that (4) the *early stages of oxidation uniformly give lighter colors than the later ones*, the series usually running from yellow to orange, through darker tints to brown or black.

Bertrand's studies make it clear that any benzene nucleus with an attached hydroxyl can be acted upon by tyrosinase and converted

into melanin pigment. Thus the whole series of compounds in the table given below (and many others besides) can be oxidized to colored compounds. On the other hand, phenylalanine, phenylmethylamine, phenylominoacetic, phenylpropionic and phenylacetic acids, alanin, glycocoll, etc., give no coloration whatever. The size, complexity, and nature of the lateral chain has only a subordinate influence ; if it is not very strongly acid or basic it will not interfere with the oxidation. Thus for example, ethyltyrosin, chloracetyltyrosin, and glycyltyrosin were oxidized with ease. The nature of these side chains does, however, *considerably modify the colors produced* by the oxidation. Neither of the three last named bodies (tyrosin compounds) give a final black color ; they begin with orange or yellow and end with red or mahogany (chocolate ?).

In order to further appreciate something of the variety of color which may arise from a *single chromogen*, and to get an introductory idea of the number and variety of chromogens to be found in the animal body, careful reference to Table I. should be made. *And here it is of the highest importance to see that a single chromogen acted upon by a single enzyme* (so far as all chemical experience has detected) *produces several colors depending upon the degree of oxidation involved.*

In regard to the rate at which these colors appeared the author's statement may be cited that in a 20 per cent. tyrosinase (80 per cent. strong tyrosin) solution, tyrosin developed a rose color in ten minutes and its black color in four to five hours.

TABLE I. (From Bertrand, '08.)

Name of Body.	Colors Produced by Oxidation.
Tyrosin	red grenadine, then inky black.
p-oxyphenylethylamine	red grenadine, then black olivaceous.
p-oxyphenylmethylamine	orange yellow, orange red, maroon.
p-oxyphenylamine	orange, mahogany red, then brown.
p-oxyphenylpropionic acid	orange yellow, grenadine red, brown.
p-oxyphenylacetic "	yellow, orange yellow, then brown.
p-oxybenzoic "	(weak) rose, orange, then yellow.
p-cresol	yellow, orange, then red.
Phenol	yellow, orange, red, then brown.

These are, from our present point of view, the more notable results of Bertrand's investigations of what he calls " the mechan-

ism of melanogenesis." This author does not in any way consider the bearing of his findings on Mendelian descriptions of the mechanism of the origin of melanin *color characters* (neither on any aspect of inheritance). Nor — as previously stated — has anyone, at any time, inquired as to whether the facts obtained in the former sphere are compatible with the assumptions made in the latter. Bertrand's studies had other and quite different objects; these were: First, to learn the degree of specificity of the enzyme tyrosinase; the conclusion here being (p. 387) "that the results speak once more against the principle of very absolute specificity which one nowadays often hears applied to enzyme actions." It will be very well for us to bear in mind this result, since, as we shall see, Mendelian description demands a still higer degree of enzyme specificity than the philosophy of biochemists has yet dreamed of. A second object of his work seems to have been to consider the possibility of identifying certain of these tyrosin bodies by the color reactions they give upon oxidation with tyrosinase. A third purpose of the study was concerned with the causes of the rather wide variations in the elementary composition of different melanins; he believes that the results give some reason for believing that the simplest melanins arise from the oxidation of tyrosin itself, while the more complex ones — those containing sulphur or iron — are formed by the oxidation of less complete products of protein hydrolysis — namely, tyrosin-containing di- or polypeptids.[1] A fourth and final phase of Bertrand's investigation touched upon a hitherto unrecognized, but seemingly possible, mode of union between tyrosin and other amino acids.

It will now be well to briefly sketch a few facts obtained from the study of abnormal tyrosin metabolism, and from pathological pigmentations (melanin) of the human body, as supplementary to the account of melanogenesis which is given above. These facts will also serve to illustrate the dependence of tyrosin oxidations upon somatic conditions which may be of such a temporary, intermittent, quantitative, or reversible character as to preclude

[1] In the formation of melanins, condensations as well as oxidations occur, but the former process need not concern us in treating the present theme.

the possibility of accounting for them on the basis of specific, independent transmissions, once for all segregated by the germ cells.

Rather fortunately for the completer view of our present theme, pathologists and clinicians have been frequently confronted with cases of incomplete tyrosin oxidation (alkaptonuria), and unusual and pathological melanin pigmentations (melanotic tumors, Addison's disease, ochronosis) in the human body. These subjects because of their medical bearings have received a very great amount of attention, of accurate and searching study, at the hands of investigators. It seems self-evident that the student of melanogenesis should here find much data to interest him.

In the condition known as "alkaptonuria"[1] the alkapton acids — uroleuic and homogentisic — appear in the urine. The last-named compound represents a stage in the oxidation of tyrosin. The intermediate stages and the chemical structure of these several compounds may be best understood by reference to Table II. Our interest in these early stages of tyrosin oxidation is very great since we know that the same, or similar steps, lead in special cases to the formation of melanin.[2] Neubauer ('08) has very recently determined the exact course of the first four steps of tyrosin oxidation as they occur in the living (human) body. The chemical expression of these stages is given in the table.

Garrod ('02) found that certain individuals, who in their youth excreted urine containing homogentisic and urolencic[3] acids, produced only the former during adult life. Other cases of temporary and intermittent alkaptonuria are known. Here certainly the evidence of our senses is simply that the *power of the organism to oxidize tyrosin compounds is not dependent primarily upon germinal segregations*, but rather upon tissue activities, relations

[1] Résumé and literature by Falta, *Biochem. Centralb.*, 3, p. 174, 1904. See also Abderhalden, "Lehrbuch der Physiologischen Chemie," Berlin, 1906, pp. 294–298; and Neubauer ('08), *loc. cit.*

[2] A chemical research directed to the determination of the reasons for some tyrosin oxidation leading to melanin formation, instead of to the usual end-products of oxidation — NH_3, CO_2, H_2O, etc., would be of the greatest interest and help in studies on the physiology, development and heredity of color.

[3] According to Neubauer ('08) this body is not an intermediate step in the oxidation of *tyrosin* to homogentisic acid.

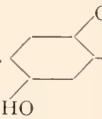
Table II.

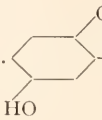
Showing Some of the Known Facts of Chemical Experience Concerning the Succession of Oxidations of Tyrosin and Closely Related Bodies to Melanin Pigments.

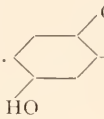
Tyrosin = ...HO—  —$CH_2CH(NH_2)$—COOH[1] Colorless.

P-oxyphenyl-pyrotartaric acid = HO—  —CH_2—CO—COOH[1] "

Chinol = ... O=  —CH_2—CO—COOH[1] "

Hydrochinon-pyrotartaric acid = ...  —CH_2—CO—COOH[1] "

Homogentisic acid[4] = ...  —CH_2—COOH[1] "

Gentisic acid = ...  —COOH "

Melanogen .....( ) ..... "

Melanin.....( ).....(White)?[2]

Melanin.....( ).....Pale yellow.[3]

Melanin.....( ).....Deep yellow.[3]

Melanin.....( ).....Red.[3]

Melanin .....( ).....Brown.[3]

Melanin.....($C_{50}H_{58}N_8SO_{12}$).....Black.[3]

Melanin.....($C_{45}H_{78}N_{10}SO_{20}$).....(White)?[2]

and conditions; these may vary or change from year to year—a power of oxidation not possessed by the individual during many early years of life being attained at manhood, or vice versa. Bateson ('02) has seen fit to claim that (p. 133, note) alkaptonuria is the result in inheritance of the union of "two recessives." Garrod has concurred in the view. But in the light of the inconstant and intermittent character of the phenomenon, it seems necessary to draw a directly opposite conclusion.

[1] This according to Neubauer ('08).

[2] See discussion of Spiegler's work ('03), etc., p. 328 of this paper.

[3] These from Gessard ('03) and from Bertrand ('08).

[4] Concerning the formation of homogentisic acid from tyrosin in plant tissues, see Schulze and Castoro, *Zeit. f. Physiol. Chem.*, Bd. 48, p. 396, 1906.

Examples of other temporary or intermittent oxidative powers might be much extended to include cases of glycosuria, cystinuria, purin metabolism, etc. I shall not discuss these cases which have only an indirect bearing on our question of tyrosin oxidation. It is, however, of some interest to state that it has become evident through the work of Abderhalden and Schittenhelm ('05), Garrod and Hurtley ('06), and others, that the body may possess a low oxidizing power for *several different protein constituents at the same time;* as, for example, in some cases of cystinuria, when diamines, tyrosin, lysin, etc., in addition to cystin, pass through the body unoxidized and appear as such in the urine.

The known facts of abnormal pigmentations deserve a larger share of attention than they receive here. They are mentioned chiefly to direct attention to a field of facts that are quite completely ignored in our theories of the heredity of color.

In the condition known as *ochronosis*, certain cartilages (*e. g.*, those of the ear) and connective tissues become pigmented. The work of Albrecht ('02), Osler ('04), Pick ('06), and others, make it certain that ochronosis is a form of melanotic pigmentation, and that it is not uncommonly associated with melanuria, or alkaptonuria and even with the pigmentation of the sclerotics and skin (Osler). Similarly, in *Addison's disease* there is deposited in the skin a pigment which, according to Pforringer ('00) differs from that produced normally only in quantity and not in origin or composition. It is well known too that *nerve lesions* are often accompanied by pathological pigmentation of the skin.[1]

A word in regard to *melanotic tumors*. These are known to occur particularly in white horses. The amount of melanin produced is often very great. Abel and Davis ('96) estimate the melanin of the entire skin of a negro at 1 gram, whereas Nencki and Berdez ('85) found 300 grams of melanin in a sarcomatous liver, and estimate that the entire body contained 500 grams.

These several facts from pathology are significant in that they indicate that *for the building of any melanin at all, the actual local conditions of the organism, or the organ, have a rôle to play that*

[1] See résumé by Schmidt, *Ergeb. der Pathol.*, Bd. III., Abt. 1, p. 551, 1896.

is quite out of keeping with any "once for all determination" by the shuffling of color "factors" through the germs.

In this connection attention should be called to the fact that Spiegler ('03) has reported the finding of a *white* melanin in the white hair of horses, and in sheep's wool. It would seem that the melanin isolated by him represents a more advanced stage of oxidation than does black melanin. If this is true it is obviously an important fact. The isolation of a similar pigment from the white hair of *albinos* seems, however, neither to have been sought for, nor found; but it is quite possible that such a pigment also exists in mammalian albinos. There are nevertheless some reasons — chiefly biological rather than chemical — for believing that among birds, at least, a white color exists which represents a less advanced stage of oxidation than that in any other of the melanin color series; in fact, here the "white" seems to be a purely "physical" color. The chromatophores do not develop, and no white pigment is histologically discoverable. Apparently, therefore, the oxidation of tyrosin, etc., is here not carried far enough to produce any color whatever.

The actual facts regarding the "white" pigment or color of birds and mammals are not yet clear. "White" forms at present a most awkward, and at the same time a most interesting gap in our knowledge of the melanin series. The elementary formulas for the black and white pigments given in the table are Spiegler's findings for the white and black hair of the horse. Certain phases of Spiegler's research have been confirmed by Wolff ('04), but the particular facts which we have referred to above have not been reviewed or confirmed.

We are fortunate in having, from physiological experiments with melanin pigments in living animals, some facts which confirm the data from chemistry and pathology regarding the mechanism of melanin production. On this point special attention may be called to the recent work of Gustav Tornier. His work furnishes a splendid view of the *control* of the color of the integument. The colors of Amphibia, from larval stages to old age, were determined at will by controlling the physiological state, particularly the nutrition, of the animals. When the color

phenomena observed by Tornier are viewed in the light of the work of Bertrand, it seems certain that in these two sets of phenomena we are really dealing with the same facts.

Tornier found that tadpoles divided into lots for differential feedings gave (1) little or no pigment in the ones fed the minimum, and progressively more pigment as the maximum is approached; (2) *a series of colors: white, yellow, red, gray, black.* Tornier concludes ('07*b*, p. 288): It is possible, therefore, by adjusting the dosage of fleshy food to force the epidermal coloration of *Pelobates* larvæ (he elsewhere describes this as true for other amphibia) into white, yellow (see p. 285), red, gray, black.[1] It will be seen that the types of color and the order of their appearance in the organism — when we put these organisms into such conditions as will force them to do the work of pigment formation (oxidation) in stages — closely follow the lines of our purely chemical experience. Tornier produces entirely comparable effects upon the coloration by two other means, viz.: by removing more or less yolk from the vegetative pole of the egg through an opening made by a needle; or by coagulating *in situ* a part of yolk proteid by the introduction of water; such coagulations of yolk proteid cannot be digested by the developing embryo. The three methods employed all reduce the nutrition of the animals, and produced albinism, erythrinism, blackness, or melanism, depending upon the state of nutrition.

It was further found ('07) that the experiment could be carried out in the *opposite direction* as well; that is to say, the highly fed, black-containing, and black-producing larvæ of large or small size could be made, through a diminution of feeding, to produce a *series of colors in the order of: black, brown, red, gray, white.* Certain observations by Powers ('08) are confirmations of Tornier's [1] results.

Without extending these illustrations it can be stated that if these facts and experiments mean anything they mean that in an animal that produces melaninic color, *there exists all the machinery necessary to produce a series or scale of these colors.* And that

[1] The proof that all these shades of color in Tornier's tadpoles were melanins is hardly as complete as is desirable, since lipochromes and traces of guanin are also known to develop normally in late larval and adult stages of these amphibia. Many facts, however, indicate that the colors here described by Tornier are true melanins.

what is actually produced is, in several demonstrated instances, *dependent upon the physiological state of the organism.* Or, perhaps, in certain cases it may be possible to say more definitely that the *limiting factor* is none other than the available oxygen or food-supply. I have been able to prove ('08*a*) definitely that in many birds the daily nutritive changes which accompany the low blood-pressures occurring at night influence the *quantity* of melanin produced.

The specific color of an animal then is an index, not of the presence in the germ from which this animal arose, of certain chromogens and specific zymogens, and the absence of a wide series of others; but, this specific color means that a *process* with a wide range of possibilities, *because of a particular physiological state and environmental conditions* has struck this particular equilibrium. *One and the same organism has within it all that is necessary to move that equilibrium up or down* — taking the red form for example, we can in the words of Tornier "force it to black or to white."

Tornier did not consider the relation of his results to any of the facts of the chemistry of melanin, nor did he consider their bearing on color inheritance. His chief concern has been apparently to establish two points in color physiology; first, the effect of varying degrees of nutrition on the size, shape, color-production, etc., of chromatophores; and, secondly, a defense of the thesis that the pigment granules of these chromatophores act as reserve food-materials in cases of inanition, etc.

Mendelian Description.

It is now possible to consider whether Mendelian interpretation and description is in accord with the facts of color formation. The Mendelian position can be best presented in the words which Cuénot ('03) used in the original formulation and statement of it. I quote practically the whole of his discussion, and ask that it be remembered that it is almost entirely upon this slender basis that the "presence, absence" hypothesis, and consequently a great share of Mendelian nomenclature, rests:

"Again one learns that the authors who have recently studied the origin of melanin pigments, Biedermann, v. Fürth, Schneider

and Gessard, state that these pigments result from the action of an oxidizing enzyme (tyrosinase) upon a chromogenic substance ; there are good reasons for supposing that things happen similarly in the pigmentation of the skin; there should be, however, in this case, either two different chromogens and only one enzyme, or only one chromogen and two enzymes, the one for the blackish pigment and the other for the yellow pigment. We adopt provisionally, for convenience of language, this latter hypothesis.

" The germ plasma of a gray mouse should contain potentially the three substances which, by their reciprocal reactions later produce the deposition of pigment in the hair; and doubtless these three substances are contained in the potential state within many of the material particles of the germ plasma (representative particles or qualitative substances of the egg — mnémons). In a gray mouse (black and yellow pigmented) there are three mnémons, one for the chromogen and two for the two ferments ; in a black mouse there are only two mnémons, one for the chromogen and another for the formative enzyme of black pigment.

" In regard to albinos, all is explained if we admit that their germ plasma contains only the mnémons of the enzymes, that of the chromogen being totally absent. With these conditions, colored hair cannot be formed in albinos, since one of the substances indispensable to the reaction is absent, but one easily understands that the albino will transmit to its progeny either the mnémons for the two enzymes, or one mnémon only, if it possesses but one."

The Mendelians have one further point to confirm the faith that is in them. Soon after the appearance of the paper by Cuénot, Durham undertook to find whether in the skins of black, chocolate, yellow and albino mammals there is the appropriate enzyme in each for the production of its particular color — when this acts upon a tyrosin solution. Only a short preliminary statement ('04) of the results has appeared ; and although positive results were reported for the black, chocolate and yellow pigments, it is evident that from no point of view can these results be regarded as satisfactory; particularly because the extracts used by her are stated to have had a *reddish* color before

adding the tyrosin; an adequate account of the history or nature of color changes in such a solution seems hardly possible since as Table I. shows much of melanin production results in colors which are paler than red and the origin of all these, and to some extent the final color, would be obscured by the initial presence of red. Miss Durham was unable to state exactly what the extracts of the albino skins were capable of doing, but thought they probably contained no such enzymes.

The factor hypothesis of Castle avoids some of the pitfalls of the earlier theory, but seems to rest on essentially the same base. It is necessary to examine in detail the statements and conclusions in Cuénot's paper.

Cuénot says: "There should be, however, in this case either two different chromogens and only one enzyme, or only one chromogen and two enzymes, the one for the blackish, the other for the yellow pigment." The facts of the origin of melanin do not substantiate Cuénot's hypothesis because the colors do not form on this plan; one and the same chromogen is known to form yellow and black; one and the same ferment — tyrosinase — is known to produce both yellow and black from the same chromogen.

According to Cuénot: "The germ plasma of the gray mouse (black and yellow pigment) should contain potentially the three substances which, by their reciprocal reactions, later produce the deposition of pigment in the hair; and doubtless these three substances are contained in the potential state within many of the material particles of the germ plasma (representative particles, mnémons, etc.)." But three such substances are not required for the production of black and yellow; these two color compounds are known to be but different stages of oxidation of the same substance. Furthermore, the locating of all the factors which determine the development of these colors in the germ plasm does not reckon with the facts of color physiology already cited.

Continuing, Cuénot says: "In a gray mouse (black and yellow pigmented) there are three mnémons, one for the chromogen, and two for the two enzymes; in a black mouse there are only two mnémons, one for the chromogen and another for the formative enzyme of black pigment." Statements made above furnish a sufficient refutation of this conception of Cuénot's.

Cuénot's concluding statement: "In regard to albinos all is explained if we admit that the germ-plasma contains only mnémons of the enzymes, that of the chromogen being totally absent. With these conditions, colored hair cannot be formed in albinos, since one of the substances indispensable to the reaction is absent, but one easily understands that the albino will transmit to its progeny either the mnémons for the two enzymes, or one mnémon only if it possesses but one." To this statement must be opposed first the opinion of Durham that the skins of the albino mammals studied by her contained no tyrosin-oxidizing enzymes; a second much more weighty and conclusive objection is that the absence of melanin chromogens in albinos is practically inconceivable. It is now certain that tyrosin and its many related compounds are such chromogens, and that these compounds have a distribution in the universe almost or quite co-extensive with protoplasm itself. *The postulation of the formation of chromogen-containing, and non-chromogen-containing gametes is therefore reduced to an absurdity. It is moreover quite certain that the food of the albino mouse must daily bring it quantities of chromogens, even if such could have been excluded from the germ cells. There is no doubt and no middle ground here. Cuénot's conception fails completely.*

Space does not permit a discussion of the facts and interpretations of the inheritance of coat colors of mice since the work of Cuénot; a subject which has been investigated or discussed by many workers, notably by Bateson, Allen, Cuénot, Morgan, Wilson, Castle and Durham. A detailed consideration of these results is omitted also because the facts are well known and do not belong here. The behavior of the color yellow first reported by Cuénot ('05) is, however, of such unusual interest as to deserve special mention. It was found that yellow is "dominant" in mice, whereas elsewhere in animals it is usually "recessive" to black; this is an unexpected result from the Mendelian standpoint, and the difficulties which it presents have called forth several highly complex, supplementary Mendelian hypotheses. It seems not to have occurred to anyone that yellow may be a *blend* formed from the union of other colors, *e. g.*, between albino and black. A glance at our scale of colors (p. 326) sug-

gests that such may be a true, and, indeed, the truest conception of a color blend; and an examination of the experimental results gives considerable support to this view. Castle ('06) reports that he secured yellow mice from three sources, viz.: Y♂ × Ch ♀, A × B, A × Ch. It will be observed that each of these crosses has one color (yellow, chocolate, black, albino) *more* oxidized than yellow, and one *less* oxidized than yellow (see Table II.); that is, the yellow produced in these cases is apparently a blend.[1] The general fact of the unstable (heterozygous) character of all yellow mice is, quite possibly, evidence of the same kind.

Heretofore a blend, say between white and black, has been considered a mixture of these two colors, a spotted animal, a form in which black was diminished, etc., but a little reflection upon facts already stated concerning the nature of these color characters reveals *very distinct colors as none the less very distinct blends!*

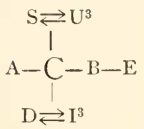
At present the *biological* data are wanting to quantitatively seriate all of the several colors; but there is apparently enough data to warrant the definite statement that *yellow mice* are forms with the power to oxidize tyrosin compounds to an *intermediate point.* Thus the biological data again parallel chemical experience.

Cuénot, Bateson and others "explain" color inheritance on the assumption that "recessives" lack altogether the *factors* of color production; but Castle has convincingly argued that this cannot be true, because in such forms *small amounts* of pigment actually form, etc. Castle tried to explain these phenomena upon the supposition that all of the factors may be present, but that "the presence of one character often inhibits the activity of another," *i. e.*, upon grounds of activity and latency. I would urge that we are now quite ready to take the next step, which seems to lie in the opposite direction, and say that we have to do neither with absent factors nor with the inhibition of present factors; that in gametic unions we deal not at all with "factor" particles, but merely mix, and amalgamate to various degrees, powers of tyrosin oxidation; and the conditions supplied by the differentiation of

[1] Cuénot says of his yellow mice that they contain numerous unfixable variations, not hereditary, ranging from a clear orange yellow to a sooty or grayish yellow, not very different from the color of gray mice. Does this look like purity of gametes, or a wide range of blending, which?

tissues and organs, together with environmental conditions external and internal, supply whatever else is concerned in color production.[1]

In his work on mice Castle ('06) shows conclusively that in these forms he is not dealing with the *total absence of a character*, even in some cases where this seems apparent; he there states that the *purity of gametes* does not exist, and further, "no more does the *purity of factors* exist." It would seem that this paper by Castle is one of the best, if not the best document extant to convince that no such things as *factors* exist. Yet, quite recently,[2] Castle has carried the factor hypothesis to its highest state of complexity, to what is apparently its logical conclusion, if any "particle" basis whatever could be granted for hereditary processes. Castle pictures his conception of the factors in melanin color inheritance and their relations in the following diagram, modelled after a chemical formula:



C = Color, or chromogen factor.
A = Agouti factor.
B = Black "
D = Dilution "
E = Extension factor.
I = Intensity "
U = Uniformity "
S = Spotted "

This visualizes for us the body of factors which are to be shuffled in the germs and "determine" the colors of the progeny. From what has been said it is obvious how far this conception leads us astray; because this complex Medelian interpretation and description of color inheritance leads us away from a simple series of color developments due to the difference in degree to which one substance may be oxidized.

The placing of the "uniformity-spotted," "intensity-dilution," and "extension" factors in these germs is a virtual surrender of the whole theory of discontinuous variation; and in reality puts

[1] The many and accumulating additions, qualifications, "contaminations," "latencies," etc., that have been attached to Mendel's ('65) original conception of dominance and recessiveness, or to Cuénot's ('03) presence and absence of a factor hypothesis, are but so many direct admissions that the "purity of gametes" conception is an error; they are but so many secondary and tertiary hypotheses of completer preformation made to bolster up a primal preformation hypothesis. Incidentally, they give present-day students the opportunity to see the child of Weismannism recapitulate the developmental history of the parent.

[2] Darwinian lecture, Baltimore, January 1, 1909.

[3] Either one of the pair may be present (or active).

the Mendelian who accepts this terminology in no position to deny the development of the melanin series on a basis of closely graduated powers of tyrosin oxidation, and so without any basis on particles or factors, whatever. It is, moreover, as plain as it is certain, that this *degree of oxidizing power* covers several of these factors (A, B, E, I, D) which are thus reduced to one; while the kernel of this formula the C, or chromogen, goes out entirely as a factor, *i. e.*, something capable of being shuffled in the germs — since as we have pointed out, such a chromogen is universal in protoplasm.

Discussion.

If the later oxidations — those which produce color — of the tyrosin compounds are each individually controlled by a separate, specific enzyme, why are not the several earlier oxidations of the same compounds similarly conditioned? If assumption will give us the whole series of tyrosin oxidations only on condition of their production by means of separate and distinct enzymes, why should it hesitate to put the whole vast array of oxidations of all aromatic, or even of all organic compounds on a similar basis — a separate and specific enzyme, separately heritable, for each step in oxidation? This is certainly not true.

It is impossible at present to announce the limits to the specificity of enzymes, and of the oxidases generally; but it can be said that it is pretty generally conceded that the oxidases present less specificity than do the digestive enzymes (see Wilcock, '06). That which weighs most heavily against the Mendelian assumption of a high specificity of tyrosin oxidizing enzymes is, however, the result of Bertrand's special study of tyrosinase which indicates no such specificity (quoted p. 324 of this paper).

But granted the greatest possible specificity of these enzymes, the Mendelian description of color inheritance becomes even more untenable; for Gessard and Bertrand have shown that "black" is the end-result of a series of successive oxidations and this final color can be attained only by having *all* of the intermediate stages actually attained. This means that the animal that transmits the enzyme for black, *i. e.*, produces black colored offspring, must *at the same time* transmit also the enzyme

for *brown, chocolate, red, yellow, etc.* (more accurately, an enzyme for each step of oxidation from tyrosin to black melanin), *without the absence of a single one.* If there be introduced here the primal Mendelian conception of the freedom of *each of these* factors to be distributed in gametogenesis according to the laws of chance, how often then may we expect pure-bred black parents to produce black offspring?[1]

There are reasons, derived from our general knowledge of oxidizing enzymes, why this assumption of high tyrosinase specificity is highly improbable and some evidence against this position has been cited from Gessard and Bertrand; a direct refutation of it is furnished by the experiments of Tornier. He showed that he could take animals which would have, according to Mendelian assumption, the enzyme for "black," and make them produce any one of three or four of the less oxidized members of the color series; these same forms could again under other conditions be made to produce the more highly oxidized black, etc. Obviously, the presence of a black-producing enzyme did not determine the color here; but conditions of life did so determine. The further assumption of inhibiting factors ready at all points of color-production to account for lower grades of color formation, and all other secondary assumptions to support the primary one are clearly unnecessary, and since a clearer, saner interpretation is possible they need not be considered.

[1] This would be the usual or expected type of specificity if such thing should exist. I call attention to its implications merely to forestall any further thought regarding its possibility. The sort of specificity of enzymes that has thus far been assumed by the Mendelians has, however, been of a different sort; namely, that for the production of each color only one enzyme is necessary, but the enzyme which produces any particular color is specifically different from those which produce other colors; the case is not really different from an assumption that pepsin is specifically different in different, but closely related races and varieties. Unlike the case cited above, here each germ possesses only one or two zymogens, each capable of supervising the complete production of some one color, and therefore all the offspring could (in contrast to above) be provided with color. The only evidence that has been adduced for this sort of specificity is the rather incomplete and unsatisfactory results of Miss Durham already cited. All else has been mere assumption on the part of the Mendelians. But this type of specificity becomes a very unusual and extraordinary thing as soon as we find that all of the colors form in a continuous oxidation series. To cover this fact the assumption is obligatory that in melanin production, six, ten or a dozen tyrosin oxidizing enzymes are concerned; that these are all able to take the first steps of tyrosin oxidation; that they are differentiated merely by their "strength," that is, the extent to which they can carry the oxidations.

The doctrine of numerous specific enzymes,[1] then, goes the way of the doctrine of specific *chromogens*, which is so decisively settled by the work of Bertrand.[2] Remembering that the Mendelian description (by implication) of what is happening on the (color-producing) surface of the body in late stages of development is thus completely awry, we may not be surprised to find that their assumptions regarding conditions in the germ are in a similarly contradictory tangle.

Our present knowledge permits neither the realization nor the imagination of a "color factor" in the germ; not even in a simple form; much less does it grant us the very composite and elaborate picture presented by Castle.

The "production of color" is a special manifestation, in rather restricted regions of an organism, of a *general power to oxidize organic compounds, possessed, presumably, by all parts of the germ cell* from which the organism arose. With the development of the body, the specialization of tissues, there arise very new environments for the oxidative processes, producing localized changes and variations in this power. That this is so is evidenced by the fact that the living substance of the various body regions oxidizes fats, sugars, and proteids with unequal ease. There can be, moreover, scarcely a doubt that certain of these regions, owing to new structure, new environment, new conditions, are able to oxidize *different protein substances* with variable ease, and to a variable extent, and even in a different way.

When one has grasped the nature of the process by which melanin color characters are formed, there is as little necessity or truth in assuming that the germ cells contain representatives or determiners to correspond to a particular color, as there is in assuming that the vapors of the Gulf contain determiners for the depth, or distribution, of the mantle of snow which they are to

[1] Mendelians must, however, accept the specificity or non-specificity of enzymes in the production of color (they must have some sort of representative particle in the germ) and either choice leads, when critically examined, to the unqualified refutation of some of their fundamental conceptions and interpretations of heredity. The established fact that each of the melanin colors represents but a point in a line — a line which records the continuity of a continuous oxidation process — is the fact that strikes hard at the very basis of Mendelian interpretation and description.

[2] Table I. shows, for example, that at least seven of the nine compounds represented are capable of producing the colors yellow and red.

form on the northern hills. There exists, to be sure, a relation between the vapors and the heaps of snow, between the egg and the definitive character — but the one is not the other, does not *contain* the other.

In accounting for melanin color characters, I would maintain that — granted the continuance of other life and developmental processes — we can account for all the major happenings of color development and inheritance with extremely little of assumption. It is *known* that one oxidase is concerned. I think we need make use of but one. It is *known* that the germ possesses actively the power to oxidize amino acids. I think we need make use of nothing in the germ than just this power. It is *known* that the protoplasm of different species, of different tissues, of different parts of a cell, possess different powers of oxidizing protein bodies. It is no tremendous assumption that germ cells are not freaks of nature in this respect, and that they too have different powers of tyrosin oxidation. The fate of color characters then is bound up (1) in the union of these particular powers of the two germ cells; (2) in the origin (through other outside developmental processess) of favorable and unfavorable regions for tyrosin oxidations; and (3) in environmental conditions.

Let us now for a moment return to the matter of color-blends. The data given furnish a nearer view — practically a new conception — of the nature of color-blends in inheritance; if the position stated in regard to these color-blends is correct a little thought will convince that at the same time new light is furnished on *alternative* color inheritance; and particularly on *what is happening* in the case of the so-called alternative (Mendelian) inheritance of a color character. It can be said definitely in such a cross that it is the *power to oxidize tyrosin compounds* (a power which I believe, by no stretch of imagination, needs to be, or can be, represented by a particle in the germ, but by a general property of the protoplasm of germ cells and of tissue cells) *that is transmitted* and that here this power of *one* of the gametes *is continued into*[1]

[1] Tyrosinase has recently been found in the *eggs* of cephalopods by Weindl ('07). Similarly, several enzymes have been isolated from germ cells in recent years; in none of these cases can we for a moment suppose that these enzymes or zymogens existed as *a* particle in any one, or in each, of the chromosomes. I would, however, by no means have it inferred that I consider the presence, quantity, etc., of tyrosinase

the zygote without being very *appreciably* increased or diminished.

It may be, however, that more than one (almost certainly several for yellow) oxidation stage of a tyrosin compound presents only the one color black. It could conceivably (see scale of colors, p. 326) happen, therefore, that a certain black (low stage of oxidation) × light yellow = yellow (dominant?); but another black (highly oxidized) × yellow = black (dominant?); *and yet each of these might be true blends, i. e.*, attain to an exact intermediate stage of oxidation to the two forms crossed. Some cases of supposed dominance of color may be therefore in reality true examples of blended inheritance.

In describing color inheritance I believe that less violence is done to the known facts of color formation, and at the same time a sounder view of developmental and hereditary processes is maintained, if it be said — without delimiting terminology, and without putting a single thing into the germ except what every one knows is there, and there in the form which is stated for it — that in the union of germ cells derived from two pure color varieties, each cell brings with it a power of oxidizing tyrosin compounds, and that the union of the pair of cells may give one or more of the following results:

1. The tyrosin oxidizing power of the male cell is established (*a*) at once, or (*b*) in the next generation or later, throughout the fertilized ovum and its derivatives.

2. The tyrosin oxidizing power of the female cell is so established. (In neither of these cases do we need to postulate the continued existence of a subdued — recessive — factor or representative.) These would be so-called dominants.

3. The oxidizing power resulting from the union is somewhat more, or somewhat less, than that of either of the gametes.

in the egg as an index, measure, or determiner of the tyrosin oxidation powers of the adult. Secrets of protoplasmic differentiation, zymogenesis, stereochemistry, and catalytic action, all block for the present the tracing or predication of any such relations.

Undoubtedly enzymes have a very considerable importance in development. By focusing attention too closely upon them it is possible, however, to underestimate the importance of other, and even of related phenomena in which the *possibility* of "inherited specificity" is entirely eliminated. I refer particularly to the "autocatalysis" of such substances as oils studied by Guenthe ('07), Mathews, Walker and the writer ('08*b*), and others.

4. The result of the union is a blend; *i. e.*, an oxidizing power intermediate to that of the two gametes.

Numbers 1 and 2 represent colors at points of fairly fixed color equilibrium, as proved by the fact that individuals, varieties and species tend to stop color formation at those points; and most of the offspring of such hybrids may reasonably be expected to breed true with reference to this character, because of such stable equilibrium. Categories 3 and 4 often represent, on the other hand, colors at points of unfixed equilibrium; stages in the oxidation of tyrosin are not easily held at these points; that such points of unstable equilibrium arise in the chemical building of melanin, as elsewhere, is practically certain; this unstable condition is followed by an immediate tendency — in the second (next) generation usually — to shift to one or both of the stable points represented by the male and female condition, or to a new point.[1]

The above considerations seem to be of far-reaching applicability. They are, I think, rigorously consistent with what we know of the oxidation process, and with the various facts of melanogenesis; whereas Mendelian interpretation is consistent with neither. How much of the totality of color-inheritance is thus brought under one point of view will be at once appreciated by naturalists; while in striking contrast is the very small fraction of such inheritance that can be brought into the Mendelian system, even with all its elaborations.

In following out the implications of our conception of the state in which these color characters exist in the germ, it may be said that hybrid offspring possessing a color of easy, fixed equilibrium, mated with similar forms may usually be expected to breed true (that is, to continue this oxidizing power into their germ cells) with respect to this character. If mated, however, with another variety possessing some very different character, let us say size, which is also in very stable, fixed equilibrium, it seems quite

[1] These four types give nothing of *qualitativeness* nor of *discontinuity;* we have to deal absolutely in these initial stages with *quantity*, degree or pitch of oxidation power, and the gaps which we find in the end-result of the development of the color characters are but the cumulative, final expressions of different degrees of oxidation power, and of the fact that certain stages of oxidation are more stable — in firmer equilibrium — than others.

conceivable — almost a necessity — that each variety should at least sometimes be able to force its stable character (if these do not both rest directly upon the same process, *e. g.*, tyrosin oxidation) practically unchanged into a new combination and produce a new form — an animal with the *color* of one parent and the *size* of the other.

This clearly brings us face to face with something resembling unit characters and particulate inheritance. I can see no reason, from studies on the nature of color formation, and from the necessary deductions as to the way in which color must be transmitted, to doubt the possibility or the probability of the formation of races with new characters, or rather a race with a new combination of old characters; and with some such newly combined characters in very stable equilibrium, *i. e.*, breeding true. In fact, it is the recognition of the state in which our color character exists in the germ, that is, as a given pitch of power for oxidizing tyrosin compounds, and this not latent but active in the germ as later, that enables us to view the mechanism by which such a result is brought about.

The demonstration of the existence of such combinations of characters is, I believe, the supreme contribution of Mendelism to our knowledge of heredity. Phenomena of dominance, of segregation (?) and proportion, are but minor and special manifestations of a process much more important, general and inclusive; and which general process is, in color inheritance at any rate, the propagation and occasional quantitative modification (four types above) of oxidizing powers, with their more or less constancy of expression through settling into points of easy or fixed oxidizing equilibrium.

Our view does not, however, allow the acceptance of the unit character hypothesis without very considerable and rather radical modification. The prevailing idea among Mendelian workers has been essentially that each character, each recognizable differentiation, each member of a group of factors that forms a character, is quite separate and capable of being shuffled in the germs, and of independent appearance in the zygote. Now, as already noted, experience with the melanins drives home the point that a long series of very distinct characters have not each even *one*

representative, but *all* together have one basis in the germ — a power to oxidize tyrosin compounds, and this capable of close and continuous gradations. Many other characters, moreover, may also be closely connected genetically with the tyrosin oxidizing power of the organism. This being true, it is easily seen that what has been quite generally taken to be a "unit character" among colors cannot be in fact a "unit" of *modification.* All tyrosin oxidations, and some others as well, may be, and probably are, modified simultaneously and in corresponding manner.

The conception of "units of modification," then, which we adopt as an apparent logical necessity cannot regard as "units" the things that have been called unit characters in the past; quite certainly some unsound criteria, a false interpretation, a misleading nomenclature, and some observed facts are all back of the old conception as it has been applied to color behavior. We need not be surprised if it be found to embrace very little truth. To determine what a real "unit of modification" is, would seem to be no easy matter. Present Mendelian methods will doubtless contribute something, though not nearly all, to the complete story. For if — and there is little doubt — other definitive characters of the soma are likewise present in the germ only as varying "strengths" of rather general powers or processes, these same powers will exercise influence directly and indirectly, and to greater or less extent, in many and diverging directions during development; so that a "unit of modification" in inheritance would, in the broadest sense, include all such effects (there would probably be found all gradations of such effects). Obviously, therefore, *individual characters are by no means units of inheritance or modification.* And in any exhaustive search for such influence or modification it is quite possible that vision will sometimes be forced to cover the whole field from antibodies and immunity to size and color; from the grossest structural modification to the most delicate functional idiosyncrasy.

The question will be asked — how may those who reject the Mendelian interpretation based on representative particles, account for the segregation and proportions observed in Mendelian behavior? To undertake a discussion of this point is obviously

to leave the province of fact, with which the body of this paper deals to enter the realm of assumption and hypothesis. The fact, that in these pages we have been able to get what we are inclined to consider the clearest view that we have at present as to the state in which a character or set of characters exists in the germ, perhaps furnishes a reason why some statement may here be made in regard to the *possible mechanism of segregation.*

It has already been stated that to us the phenomena of dominance and segregation are only minor, surface, and incidental phenomena of heredity;[1] the really important Mendelian contribution being that certain different characters (such as have, according to my belief, different rather general processes as a basis) of different races may be combined to form new fixed races. The establishment of this last-named fact has been most commonly considered by Mendelians as, on the one hand, a consequence of the laws of dominance and segregation, and, on the other hand, as a strong argument for a "representative particle" basis for these two sets of phenomena. When, however, we learn that a certain character has no other existence in the germ than a rather general protoplasmic power, the "mnémon" conception fails completely and with it the supposed mechanism of its segregation — *i. e.*, the shuffling of mnémons in the reduction divisions of the chromosomes.

How then on my view of the basis of color inheritance may the segregation and proportions which result in Mendelian behavior be accounted for? I may say at once that I do not know; but in this respect I consider myself hardly worse off than the wisest Mendelian. My *supposition*, however, would put less faith than is theirs in the behavior of chromosomes during the

[1] It would seem that instead of viewing the real and entire sea of heredity, learning of its intimate nature, searching its boundaries, sounding its depths, too often Mendelians have — to indulge a figure — focused their visual instruments upon an optical section lying perhaps a few meters *above* sea-level. Here, occasionally, beautiful and regular phenomena come into sight; but for the most part the field is blank. At times great ocean swells pass in fine order and precision, permitting the observer to predict quite well some of the attributes of the next undulation and the time of its appearance; again, bits of spray or foam or mist may sometimes come into view, and the seeming disconnectedness of it all permits the be-focused onlooker to name and classify — and wonder. All — while the great ocean of heredity with its perfect continuities, its essential oneness, its inclusiveness, lies in unseen constancy and majesty beneath.

maturation divisions. It may for the present be assumed, and this is, I think, all that is really demanded in accounting for the differences in end-result in color development and inheritance — that the germ cells vary in "strength," *i. e.*, in such general powers as assimilation, growth, oxidation, etc. (and this proposition is not all assumption); this general difference of "strength" of germ cells may (or may not) arise during the maturation divisions; one or more of the four cells receiving in some species, though not in all, a type of protoplasm in better or worse condition than the others — influenced, for example, by more or less yolk; yolk more or less affected by previous nuclear and cytoplasmic contact; by variable distribution of the protoplasm of the astrosphere; by variable admixtures of nuclear (not necessarily chromatin) and cytoplasmic matter; by receiving more or fewer entire chromosomes, etc. (Chromatin is well known to be a very reactive substance and we may very well believe that wide variations of it in amount influence the vigor or intensity of vital processes occurring in a cell and in its derivatives; but in this respect chromatin is not unique, the other variables just mentioned doubtless do the same.) Any or all of these things will influence the development of a character only by serving to strengthen or weaken some *process* which underlies its formation.[1]

Such general differences of germ cells as arise from the several possible causes mentioned above would conceivably tend to affect the strength of such a general process as oxidation — such as produces scales of color. From the nature of these differences it will be seen that the growth and maturation stages may occasionally among organisms — constantly perhaps in some forms — furnish the conditions for the production of four sister sperm cells of unequal strength, one or two may be especially favor-

[1] It is perhaps well in our estimation of the basis for segregation of color characters, which rest directly on an oxidation process, to call attention to the special significance of the centrosome and cytaster. Considerable evidence is adduced by Mathews ('07) to show that the centrosome is the reduction center of the cell. If this should prove true, very obviously our estimation of the oxidation powers of the cell would be better treated in relation to this body than to any other in the cell. It cannot be too much emphasized, however, that upon this view the centrosome and the sphere is but a *region* where intense reduction occurs; the intensity grading off from centriole to (presumably) the periphery of the cell; and this region should be thought of as an expression of general powers of the whole cell.

ably equipped by the distribution of such materials, while one or two are left poorer than the others.[1] A similar conception may be applied to the ova. Then upon the union of male and female cell, two oxidizing powers of equal or unequal rank, of higher or of lower degree, meet. A stable (pure breed) high equilibrium results in, say one of four; a stable (pure breed) lower equilibrium results in another; while in the other two perfect equilibrium is not at once attained.

Here is then a *possible* picture of the basis of Mendelian segregation and proportion, but without recourse to hypothetical "particles" or to immutable and immortal factors. An apparently very specific end-result of an oxidation would be traceable in the germ only in the strength or pitch of a general vital process, and not at all in mnémons or representative particles packed with unthinkable precision, order and potentiality into (presumably) the chromosomes. But the above is a possible picture only; and it is not here my purpose to furnish nor to defend at length a possible or probable theory of the mechanism of heredity. The material in hand lends itself first of all to the demonstration of the *impossibility* of many Mendelian views.

The nature of present Mendelian interpretation and description inextricably commits to the "doctrine of particles" in the germ and elsewhere. It demands a "morphological basis" in the germ for the minutest phase (factor) of a definitive character. It is essentially a morphological conception with but a trace of functional feature. Although heredity is quite surely a functional process of major complexity, it may be recalled that the primary and fundamental Mendelian conception of this process utilizes not a single finding of the science of biochemistry; that the only physiological fact utilized is the one of certain occasionally observed segregation behavior which is exhibited in the end-results of varietal[2] or specific character formation; such segregation, by

[1] We know that in the corresponding divisions of *ovogenesis* that extremely disproportionate distribution of yolk and cytoplasm occurs quite constantly. We may believe that the laws which there give rise to the *extreme* differences between polar body and oöcyte are not *everywhere else*, and *completely*, unoperative; they may be operative — though in a much less pronounced degree — in providing different *mature* ova, and different sperms with varying amounts of the materials mentioned above.

[2] De Vries ('01, '05) has asserted that only varietal, not specific, differences exhibit Mendelian heredity; this statement is not accepted by Bateson ('06), and is controverted by still other workers.

inference, arising from the temporary mixture (heterozygote), or failure to mix (homozygote) in the gamete, of something, no one knew what — but which has been generally conceived of as some sort of "particles." (In *later* additions to, and *special* applications of the Mendelian conception, certain other biochemical and physiological facts have, of course, been considered.)

This is precisely why present Mendelian interpretation and description of heredity is a bar to the progress of studies in inheritance and development; with an eye seeing only *particles*, and a speech only symbolizing them, there is no such thing as the study of a *process* possible.

The conception that organic color has at its basis not rigid, immortal particles, but yielding, equilibrium-seeking powers, or strengths of processes, makes the infinite variety of colors in organisms intelligible. If, on the other hand, particles, and a mechanism for their continual segregation and propagation pure were in reality at the basis of color inheritance, we should rather expect uniformity, not the actual diversity, to be the dominant feature of organic coloration. Indeed, a modification of the strength of many organic processes (and so of color formation) would be a necessary accompaniment — a result, even if not a cause — of that "transformation of organs" which has been the very labor of phylogenetic development. The atrophy, superior development and transformation of organs are certainly efficient factors in such modification, for it is a physiological fact of common experience that many, or most, of the vital processes are not equally strong or pronounced in all of the organs of the same organism; and that many metabolic processes of the body are dependent upon special organs for their highest expression, for the completest manifestation of their power.

Let me not be understood to say that our knowledge of the development of melanin color characters is complete. There is much yet to be learned. But the significant thing about it is that we now know *so much* of the mechanism of the building of these characters in comparison with what we know of a similar nature in non-color characters. It has been possible, I think, to show by means of what we know of the genesis of these color characters that the Mendelian description — of color inheritance

at least — has strayed very wide of the facts; it has put factors in the germ cells that it is now quite certainly our privilege to remove; it has declared discontinuity where there is now proved continuity; it has postulated preformation where there is now evident epigenesis.

Is it too much to expect that the further application of such tests as the one here presented in outline for the melanin colors will in the end remove *many* of the Mendelian "factors" from the germ cells? That many of their "characters" will come to rest on a more proximate basis; will be known to have their "determination" and origin in very general germinal powers, and in somatic conditions obtaining previous to, or at the time of, their development? Will not other Mendelian discontinuities then begin to disclose gradations, and other qualitative differences then appear more and more as quantitative sequences?

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